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# Physiological Zoölogy

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# PHYSIOLOGICAL ZOÖLOGY

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Physiological Zoölogy

*Vol. IX* JULY 1936 *No. 3*

# DIFFERENTIAL ACCELERATION IN FROG DEVELOPMENT

(Eight figures)

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ON THE theory of axial gradients, a stimulating agency applied in early development should steepen the gradient and exert a differential acceleration upon apical structures, just as toxic agencies should flatten the gradient and exert a differential inhibition. Of differential inhibition numerous examples are known. For differential acceleration, however, the evidence is less full and mostly applies to invertebrates (for examples of this and of differential inhibition see Child's books [1915, 1924]). The vertebrate cases are confined to the effect of atropine on the fish *Macropodus* (Gowanloch, 1924) and those of weak acids and extremely weak toxic agents (by differential acclimatization) on frogs, reported by Bellamy (1919, 1922).

Gowanloch's work has unfortunately never been published *in extenso* and cannot be evaluated. Bellamy found definite acceleration of rate of development by the application to early stages of very weak concentrations of various normally toxic substances. He especially notes n/500 NaOH, 8 days from early gastrula; m/100,000 KCN, 4 days from fertilization; n/5,000 and n/20,000 HCl for 9 days from early cleavage. This absolute acceleration was accompanied by the formation of relatively large heads (differential acceleration in Child's sense).

Professor Child, in a letter, suggested the use of pilocarpine. This had been found by Matthews (1902) to accelerate development in sea urchins. Sollman (1904), working with sea-urchin and starfish eggs, confirmed this accelerating effect for low concentrations of pilocarpine, though high concentrations retard. Atropine in low concentrations has no effect; in higher concentrations it has a marked retarding effect. Pilocarpine further was found by Hinrichs (1929) and Hinrichs and Gunther (1929) to have a slight accelerating action on cleavage in *Arbacia* and a marked differential stimulative action on apical regions, whereas atropine was highly inhibitory and caffeine gave negligible results. Unfortunately, Hinrichs' paper is only a note, and gives no detailed protocols or figures. Earlier, Hinrichs (1924) had studied the effect of caffeine on *Planaria*, with especial regard to oxygen consumption, and found in general that low concentrations caused a marked increase, followed later by a decrease, in metabolism. Hinrichs' results with pilocarpine and caffeine, though scrappy, are suggestive.

In view of the great advances of recent years in the analysis of vertebrate development, we decided to repeat some of these experiments on frog's eggs (*Rana temporaria*). The present paper, though largely in the nature of a preliminary communication, records some definite results, notably with pilocarpine and HCl.

## METHODS

After artificial fertilization, the frog's eggs were placed, either immediately or sometimes in later embryonic stages, for a certain period (mostly 1–2 days) in various solutions of caffeine, atropine, pilocarpine, ethyl alcohol, potassium cyanide, lithium chloride, and hydrochloric acid, respectively. At certain stages of larval life (mostly 7–10 days and 5–6 weeks) samples of tadpoles were taken from the various solutions and preserved in Bouin's alcoholic fluid. These samples were drawn later in ventral and in lateral view on millimeter paper with the aid of a projection apparatus (constructed by Mr. D. A. Kempson), which gave a magnification of exactly 12×. From the sketches the proportions of the larvae were calculated and tabulated in order to compare the effect of the various solutions upon the size and proportions of the larvae. It should be mentioned that the data obtained from the sketches were not recalculated into millimeters; thus the units given in the tables and text are 1/12 millimeter each. Five measurements were taken from each sketch, as follows (see also Fig. 1): (1) maximal head breadth, as seen in ventral view; (2) maximal head length, determined arbitrarily as the distance from the extreme anterior end to the most posterior level of anterior gill insertion, as seen in ventral view; (3) maximal trunk breadth, as seen in ventral view; (4) trunk length, as measured from the anterior end of the head to the posterior level of the orifice of the anal tubule, as seen in lateral view; (5) total length, as measured in lateral view from the anterior end of the head to the posterior end of the tail, along the main axis of the body (*not* the shortest distance in cases where the body is bent).

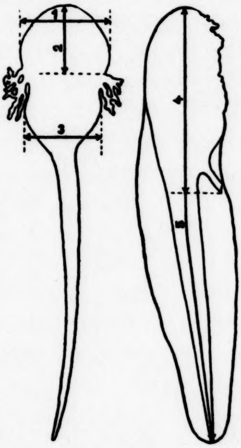


FIG. 1.—Outline sketch of 8-day frog larva in ventral (*a*) and in lateral (*b*) views, showing measurements made (indicated by double arrows): 1, head breadth; 2, head length; 3, trunk breadth; 4, trunk length; 5, total length.

A preliminary analysis of the data so obtained showed that two main ratios can be calculated from them which seem to be characteristic for the larvae. The one is the head-breadth/head-length ratio; the other the ratio of either total length to head length, or, what is essentially the same but gives more marked results, "body length" (as obtained by subtracting head length from total length) to head length. A third ratio which we first thought to be of importance, i.e., trunk length to head length, turned out to be of little value, because the trunk length shows a great fluctuating variation, which seems to bear no relation with other proportions. A few individual measurements are given in Table I.

It soon turned out, however, that the differences between the samples treated with various solutions are too slight to override individual variability. Thus, only mass methods were applicable, and the results here reported are based on averages of a number of measurements. Owing to this circumstance, not all of our sample stocks were available for analysis, but only those which contained fairly high numbers of individuals. From the three series of our experiments, the third series (Exp. 13/4/33), which was in many respects the most elaborate, has been included *in extenso;* and practically all individuals from it were drawn, except those which were obviously abnormal (e.g., larvae with tumorous outgrowths, etc.) or were damaged during preservation. In this series the effects of pilocarpine and of hydrochloric acid were studied. In addition some stocks of the first series of experiments (Exp. 3/4/33) were studied in detail, i.e., those treated with caffeine, atropine, and potassium cyanide; but the numbers preserved were unfortunately too small to allow of definite conclusions being safely drawn. The second series of experiments (Exp. 10/3/33) has been used for auxiliary purposes only.

TABLE I

| Head Breadth | Head Length | Trunk Breadth | Trunk Length | Total Length |
|---|---|---|---|---|
| KCN, m/100,000, 9-Day Larvae | | | | |
| 23.5 | 19.0 | 21.0 | 57.5 | 123 |
| 26.0 | 22.5 | 22.5 | 57.5 | 128 |
| 27.0 | 22.5 | 24.5 | 56.5 | 141 |
| 28.5 | 25.5 | 26.0 | 61.0 | 145 |
| 29.5 | 25.5 | 26.5 | 59.5 | 146 |
| 32.0 | 27.0 | 29.0 | 58.5 | 151 |
| HCl, m/5,000, 8-Day Larvae | | | | |
| 20.0 | 14.0 | 17.5 | 56.0 | 110 |
| 21.5 | 15.0 | 20.5 | 61.0 | 115 |
| 22.0 | 15.5 | 21.0 | 58.5 | 124 |
| 23.5 | 15.5 | 21.5 | 57.5 | 127 |
| 23.5 | 15.5 | 21.0 | 55.5 | 128 |
| 26.0 | 18.5 | 24.5 | 55.5 | 138 |
| 26.5 | 18.5 | 25.0 | 60.5 | 139 |

## RESULTS

*a*) *Effects on rate of development.*—Certain agents definitely accelerated the rate of development above that of the controls, while others delayed it. Delay was noted with LiCl and alcohol and the stronger concentrations of KCN. Very weak KCN sometimes caused slight acceleration. Certain concentrations of HCl and of pilocarpine caused definite acceleration. This was most pronounced with m/10,000 HCl (24 hours' exposure) and 1/4,000 pilocarpine (24 hours' exposure) (both from first cleavage); m/5,000 HCl (24 hours) and m/10,000 HCl (48 hours) were also accelerated, but to a lesser degree. This was also true for pilocarpine 1/4,000 (48 hours) and 1/2,000 and 1/8,000 (24 hours).

For some reason the acceleration caused by HCl in Experiment 10/4/33, though distinct, was only slight, and not nearly so marked as in the other two experiments. The acceleration caused by HCl and pilocarpine was visible after 2 days; but after 7 days and over it was more obvious in the HCl series, while the pilocarpine larvae became characterized by relatively larger heads (see below).

Thus a distinct absolute acceleration of development was caused by the same two agents which, as will be seen later, caused differential acceleration in the sense of a relative increase in the size of certain anterior measurements. This confirms Bellamy's work. However, as will be seen, the type of differential acceleration was not the same in HCl and atropine, so that we cannot put down the changes of proportions to a purely non-specific quantitative factor expressing itself also in acceleration of development.

*b*) *Effects on proportion of parts.*—Beginning with the most numerous data, i.e., those concerning the 8-day larvae of Experiment 13/4/33, one finds the following regularities (see Table II):

1. The normal head-breadth/head-length ratio is between 1.27 and 1.36, and the normal body-length/head-length ratio is between 6.6 and 6.7. Both the stocks kept in the weakest HCl and pilocarpine solutions give these ratios. Unfortunately, owing to inadequate numbers of the controls, no controls were preserved at 8 days: controls were, however, preserved at 7 days (see below). However, the fact that the weakest HCl and pilocarpine 7-day stocks give closely similar results, which then diverge in various particulars in higher concentrations, is evidence that these weak solutions had only slight effects.

2. The stronger HCl stocks seem to keep the normal body-length/head-length ratio (i.e., the relative head length does not vary with

## TABLE II

SUMMARY OF EXPERIMENT 13/4/33, 8-DAY LARVAE

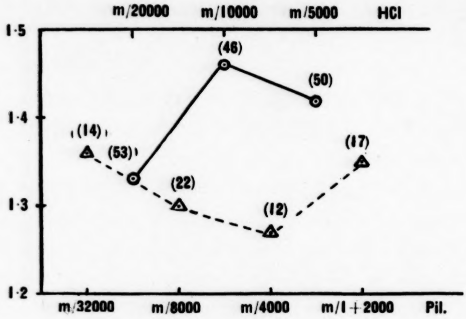
| | No. of Specimens | Head Breadth | Head Length | $\sqrt{\text{Head Breadth} \times \text{Head Length}}$ | Body Length | Total Length | $\frac{\text{Head Breadth}}{\text{Head Length}}$ | $\frac{\text{Body Length}}{\text{Head Length}}$ | $\frac{\text{Body Length}}{\sqrt{\text{Head Breadth} \times \text{Head Length}}}$ |
|---|---|---|---|---|---|---|---|---|---|
| HCl: | | | | | | | | | |
| m/5,000......... | 50 | 22.8 | 16.1 | 19.15 | 107.9 | 124 | 1.42 | 6.70 | 5.63 |
| m/10,000........ | 46 | 23.1 | 15.8 | 19.10 | 105.2 | 121 | 1.46 | 6.66 | 5.51 |
| m/20,000........ | 53 | 22.8 | 17.2 | 19.80 | 113.8 | 131 | 1.33 | 6.60 | 5.75 |
| Pilocarpine: | | | | | | | | | |
| m/1,000–2,000... | 17 | 22.2 | 16.5 | 19.14 | 101.5 | 118 | 1.35 | 6.12 | 5.30 |
| m/4,000......... | 12 | 23.5 | 18.6 | 20.90 | 107.4 | 126 | 1.27 | 5.84 | 5.14 |
| m/8,000......... | 22 | 23.6 | 18.2 | 20.72 | 111.8 | 130 | 1.30 | 6.14 | 5.39 |
| m/32,000........ | 14 | 24.2 | 17.7 | 20.67 | 118.3 | 136 | 1.36 | 6.68 | 5.72 |

HCl treatment), whereas the head-breadth/head-length ratio markedly increases in more concentrated solutions (1.42–1.46). In other words, the head shape tends to become broader under the influence of hydrochloric acid.

3. On the contrary, in the pilocarpine stocks the normal head-breadth/head-length ratio is approximately constant in the various concentrations, the fluctuations being within the limits of probable error, i.e., the shape of the head does not vary with pilocarpine treatment. On the other hand, the body-length/head-length ratio definitely decreases in the stronger solutions (from 6.7–6.8 to 5.8–6.1), which means that the relative length of the head increases with pilocarpine treatment (see also Figs. 2–7).

A measure of the size of the head as a whole may be arrived at by taking the square root of the product of head breadth times head length. The ratio of body length to this then gives a measure of relative head size. For this, the lowest concentrations of both HCl and pilocarpine again give approximately equal values: relative head size then increases slightly with greater concentration of HCl but markedly with greater concentration of pilocarpine.

Table II and the graphs (Figs. 2–7) bring out the following further points. Increasing concentrations of pilocarpine cause: (1) a progressive decrease in absolute total length (Fig. 7); (2) a progressive decrease in absolute body length (Fig. 5); (3) an increase (followed by a slight decrease) in absolute head length (Figs. 5, 6); (4) a slight progressive decrease in absolute head breadth (Fig. 5); (5) a progressive increase (followed by a marked decrease) in absolute head size; (6) a marked increase (followed by a final decrease) in relative head length (Fig. 3); (7) a definite decrease (followed by an increase) in relative head breadth (Fig. 2); (8) a marked increase (followed by a decrease) in relative head size (Fig. 4). Increasing concentrations of HCl, on the other hand, cause an irregular decrease in (9) total length (Fig. 7), (10) in absolute body length (Fig. 5), (11) in absolute head length (Figs. 5, 6), and (12) in absolute head size; and (13) no decrease or a slight increase in absolute head breadth (Fig. 5); they also cause (14) a negligible progressive decrease in relative head length (Fig. 3), (15) a definite but irregular increase in



FIGS. 2–7.—Effects of HCl (continuous line) and pilocarpine (dotted line) on 8-day larvae, experiment 13/4/33. In all figures, the groups treated with m/2,000 and m/1,000 pilocarpine have been combined, and the abscissae represent concentrations.

FIG. 2.—Effect on relative head breadth. Ordinates, head-breadth/head-length ratio.

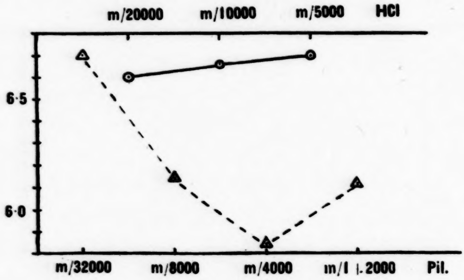


FIG. 3.—Effect on relative head length. Ordinate, body-length/head-length ratio

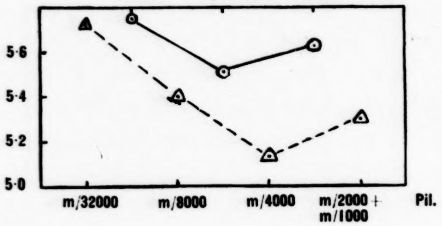


FIG. 4.—Effect on relative head size. Ordinate, body-length/head-breadth × head-length ratio.

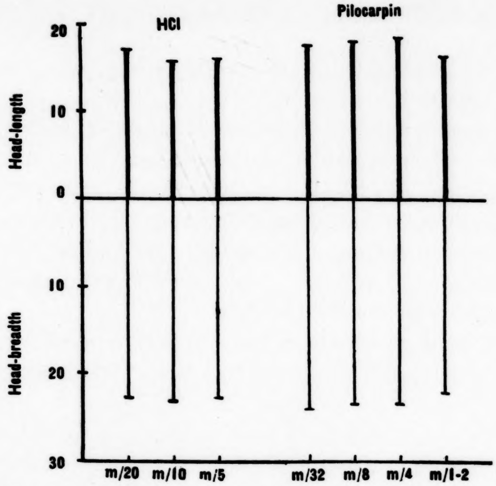


FIG. 5.—Absolute measurements of head length and head breadth. In this and Figures 6–7 the units on the ordinate each represent 1/12 mm.; and all concentrations are to be multiplied by $10^{-3}$.

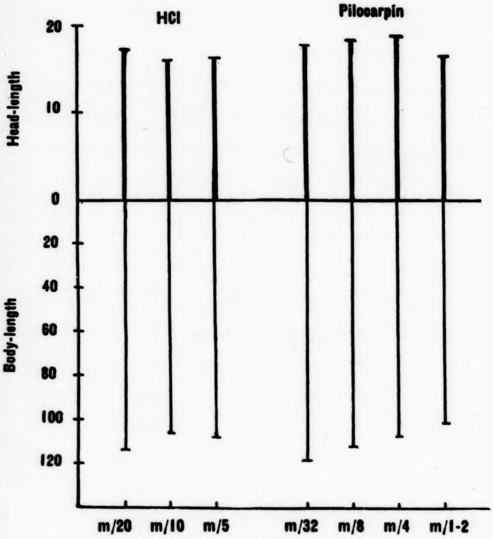


FIG. 6

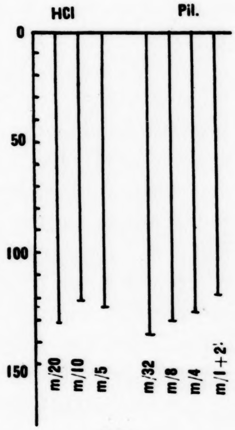


FIG. 7

FIG. 6.—Absolute measurements of head length and body length (=total length−head length).

FIG. 7.—Absolute measurements of total length

relative head breadth (Fig. 2), and (16) a similar but less marked change in relative head size (Fig. 4).

The differential acceleration exerted by pilocarpine on the head is well shown by the progressive increase of head length and negligible decrease of head breadth, the two combined giving progressive increase of absolute head size, accompanying definite decrease of body length, in the first three concentrations. The highest concentrations (m/2,000+m/1,000) appear to have been overstrong and to have begun to exert a general inhibitory effect.

Similarly, the increased head breadth in m/10 HCl accompanying decreased head length and decreased body length well shows the effect of this agent upon head growth in breadth.

Besides these 8-day larvae, small stocks (7–9 specimens each) of 7-day larvae were also preserved in this experiment. These stocks contain the most advanced larvae of various concentrations, and thus cannot be directly compared with the much more numerous and random samples of 8-day larvae. One point, however, should be noted, which is manifested by these larvae, as well as by others to which we shall refer later; this is, that in general the shape of the head alters with age. In older larvae the relative length of the head definitely increases, and thus both the body-length/head-length and the head-breadth/head-length ratios gradually decrease. This is apparently due to the formation of the internal gill system during larval life. As a result of this, we find in the 7-day larvae somewhat greater values for both the body-length/head-length and head-breadth/head-length ratios than in the 8-day larvae (6.9–7.4 as against 6.6–6.7, and 1.37–1.49 as against 1.33–1.36).

As regards the effects of chemical agencies, the results from these 7-day larvae, so far as they go, confirm those found in the 8-day samples.

Thus, the average head-breadth/head-length ratio is markedly increased in comparison with the control stock (1.49 for HCl, 1.45 for pilocarpine, 1.37 for controls). The body-length/head-length ratio is, however, inconclusive, as it is smaller in the pilocarpine but bigger in the HCl stock than in the controls (6.85 for pilocarpine, 7.03 for controls, 7.42 for HCl). It must be repeated that the numbers are too small to be conclusive. The same applies to the 5-week larvae, which were also preserved in very small numbers (4–8 in each sample) on the erroneous supposition that the differences would be clear-cut enough to be demonstrable on any treated specimen. However, it is noteworthy that in these larvae the body-length/head-length ratio is as low as 4.7–4.9, in spite of the increase of total length to 180–95 units; and the head-breadth/head-length ratio is only 1.25–1.33, which thus confirms our statement as to the change of shape of the head with age.

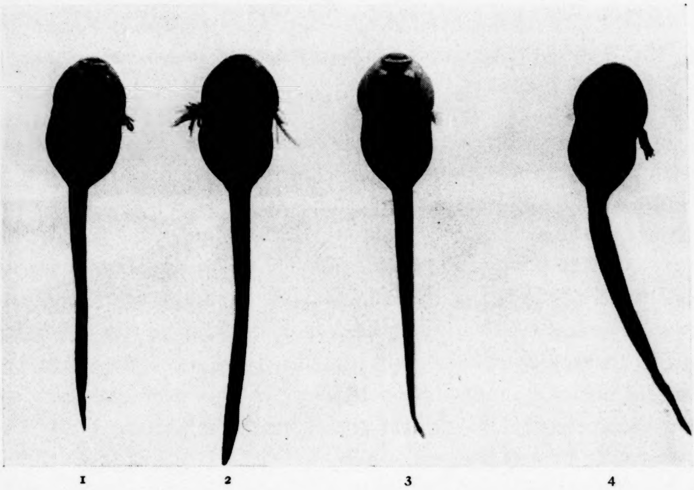


FIG. 8.—Ventral view of larvae of comparable development, showing the effect of various treatments. From left to right: 1, control; 2, treated with HCl—broad head; 3, treated with pilocarpine—large head; 4, treated with atropine—long head.

The caffeine, atropine, and KCN stocks of Experiment 3/4/33[1] were, as noted, statistically insufficient. Further, owing to higher temperatures, the 8-day larvae of this experiment were further advanced in development than those of the same temporal age in Experiment 10/4/33, making direct comparison impossible. In general, however, it may be stated that all these treatments, notably those with the weakest solutions of KCN, increased relative head length.

---

[1] Caffeine m/2,500; atropine m/10,000 and m/50,000; 24 hours from dorsal lip; KCN m/10,000 for 2 days and m/100,000 for 4 days (renewed daily) from 1st cleavage.

This is evident when samples of about the same age (as judged by the gills) are preserved and compared (see Fig. 8). Further, when the treated 8-day larvae of this experiment are compared with the 5-week larvae of other experiments, it is found that their head-breadth/head-length ratio is lower instead of higher, thus forming an apparent exception to the rule that older animals have relatively longer heads, which can only be due to these larvae having unusually narrow, long heads for their age. In the 10/4/33 series, the weaker KCN (m/50,000 and m/10,000, 24 hours from first cleavage) were also markedly long-headed, while stronger solutions (m/4,000 and m/8,000) were definitely, if slightly, inhibited in all dimensions.

## CONCLUSIONS

Apart from stating that distinct evidence of some differential action on head form is apparent, together with evidence that in some cases absolute acceleration of development occurs, no profitable discussion of the results with atropine, caffeine, and KCN can at this stage be attempted. The pilocarpine results, on the other hand, appear to be conclusive. Pilocarpine in concentrations of m/8,000 and m/4,000 exerts a differential stimulation on the head region, causing this to enlarge both absolutely and relatively, even though it produces a decrease in absolute body length. At concentrations above m/4,000 the stimulating effect gives place to inhibition, confirming Hinrichs and other workers.

The results with HCl are not so marked as those recorded by Bellamy. There is, however, a distinct change in the shape of the head in the direction of increased breadth, and a slight but definite increase in relative (but not absolute) head size. In both cases this effect is greatest at m/10,000. It should also be noted that our treatment lasted only 1 or 2 days, while Bellamy, using similar concentrations, obtained his best results by leaving the eggs and embryos in the solutions for 9 days.

It is important to recall that the treatment was applied in early stages (usually from first cleavage, sometimes from early gastrula) and that the effects only became visible much later. Either the gradient system was altered (in the egg as a whole or in the individuation field of the organism) or the activity of the organizer was modified in some other, as yet unascertained, manner.

It is proposed, now that we have gained experience as to suitable substances, concentrations, times of treatment, etc., to repeat the work on a larger scale on fish material.

## SUMMARY

1. Pilocarpine, HCl, and apparently also atropine, caffeine, and KCN, administered in weak solutions to developing frog's eggs for 18–48 hours in early stages (usually from first cleavage, sometimes from early gastrula), cause various differential accelerations in development.

2. Pilocarpine accelerates the development of the head as a whole; HCl accelerates the development of head breadth; whereas KCN, atropine, and perhaps also caffeine, seem to accelerate the development of head length.

3. The effects are clearly shown in 8-day larvae but possibly persist in later stages also.

4. In general the relative length of the head increases during larval development, owing to the formation of the internal gill system.

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# EXPERIMENTAL ASYMMETRIES OF THE HEAD OF EUPLANARIA DOROTOCEPHALA[1]

(Twelve figures)

OLIN RULON
Hull Zoölogical Laboratory, University of Chicago

THE remarkable capacity of pieces of planarians in reconstituting whole animals long ago aroused the curiosity and interest of various investigators. The result has been that many reports dealing with the regulation of pieces of different sizes and shapes from various species are to be found in the literature. In general it has been found that pieces of planarians above a certain minimum size and regardless of shape reconstitute, after a time, into perfectly symmetrical individuals, providing the species possesses the capacity for reconstitutional development.

It is true that short transverse pieces, particularly from the more posterior regions of the anterior zooid, in *Euplanaria* commonly give rise to headless individuals or individuals possessing various types of inhibited heads (Child, 1911); but this phenomenon has been shown to be the result of an inhibitory factor arising from posterior section (Child and Watanabe, 1935). In other words, all levels of *Euplanaria* will develop normal heads if the head-forming cells of the anterior cut surface are not exposed to influences arising from posterior section or other inhibiting conditions. Certain inhibiting agents have been used to block the activity of the posterior cut surface in short transverse pieces of *E. dorotocephala*, thereby inducing an increase in the head frequency at levels in which the head frequency would normally be very low (Child, 1916; Buchanan, 1922; Hinrichs, 1924).

In the regulation of pieces toward a normal bilateral symmetry it has been found that the polar relationships within a piece may be altered during the reconstitutional process. One of the best examples of this is given in the work of Beyer and Child (1930), in which it was shown that short transverse pieces of *Euplanaria*, half the width of the body or less, may develop heads on the anteromedial angle or the medial cut surface of the piece instead of on the anterior cut surface. In spite of the inhibition of heads and the alteration of polarity, the animals which reconstitute generally attain a bilateral symmetry; and it is with symmetry in reconstitution that this paper deals.

---

[1] This investigation was supported by funds from a grant by the Rockefeller Foundation in aid of research in the biological sciences at the University of Chicago.

## THEORETICAL CONSIDERATIONS

Morgan (1901) was much interested in the fact that the reconstituting head in *Planaria lugubris* (according to Hyman, 1931, this species is probably *Curtisia foremanii*) did not arise in the middle of an oblique-anterior cut surface. Instead it appeared, in its early development, to be restricted more or less to the more anterior region of the cut surface. The new head was usually asymmetrical in that the side which developed nearest the longer margin of the body gave rise to an eyespot before the more posterior side. There seemed to be even more asymmetry in the reconstitution of an animal from an oblique strip (made by two parallel oblique cuts) than in the reconstitution from a piece with only an oblique-anterior cut surface.

Rand and Boyden (1913), in working on the inequality of the two eyes in heads arising from an oblique-anterior cut surface in *Planaria maculata*,[2] noted a tendency of the inner eye to be smaller than the outer eye. It was found in this work that the animal curved toward the shorter side after the anterior end was removed obliquely. The eye which appeared on the side of the head arising on the shorter side of the animal was referred to as the "inner" eye, the "outer" eye being, of course, the eye which appeared on the side of the head arising on the longer side of the animal. They also found in a few cases that there was a tendency for the size of the eyes of the two sides to be reversed if a longitudinal strip was removed from the outer side of the reconstituting animal. The removal of this strip resulted in a reversal of body curvature, particularly in the more posterior regions of the piece. Rand and Boyden do not attempt to give a definite explanation for this temporary inequality of the two eyes, but they do say that the positional relations of the larger and smaller eye are closely correlated with the form of the regenerating piece.

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[2] Hyman (1931) would probably designate this species as *Euplanaria maculata* or *E. novangliae*.

Watanabe (1935*a*) has shown that different levels in *Euplanaria dorotocephala* develop heads at different rates. In the anterior zooid the more anterior levels develop eyespots before the more posterior levels of the same zooid. (These experiments were conducted with pieces long enough to give normal heads.) In experiments such as those described by Morgan (1901) and by Rand and Boyden (1913) with an oblique-anterior cut surface alone, it is readily seen on the basis of Watanabe's findings why the more anterior (outer) side of a head developing from an oblique-anterior cut surface should develop an eyespot before the more posterior (inner) side.

Child and Watanabe (1935), in attempting to determine the relationships between anterior and posterior cut surfaces of short transverse pieces of *Euplanaria dorotocephala*, gave considerable attention to a particular series of experiments which throws much light on temporary asymmetry in the reconstitution of a new head. They found that in the posterior half of the animal asymmetry in the head developing from the cut surface could be induced by imposing a posterior half-section a short distance behind the anterior whole section. The asymmetry of the head appeared as a temporary inhibition in size of eyespot and auricle as well as in amount of new tissue on the side of the head affected by the posterior half-section. When a single posterior operation was made, only a small number developed asymmetrical heads (19 per cent); but when the wound was reopened shortly after healing, 43.5 per cent of the animals developed asymmetrical heads. If a block of tissue was removed at the site of the posterior half-section, 81 per cent of the heads were asymmetrical. The data presented by these authors and by Watanabe (1935*b*) on *E. maculata* suggest that the posterior cut surface acts in preventing the dedifferentiation and activation of the head-forming cells at the anterior cut surface by nervous transmission of the inhibiting factor. In the making of posterior half-sections, as in the experiments above, only one of the ventral nerve cords was affected, with a resulting inhibition of only one side of the reconstituting head.

It is clear from the foregoing investigations that two factors of an entirely different nature may act in producing asymmetry in the reconstituting head of *Euplanaria*. The first deals with the fact that in the anterior zooid there is an anteroposterior gradient in rate of eyespot formation which clearly indicates why an oblique-anterior cut surface with different head-forming rates along its expanse should give rise to an asymmetrical head. The second deals with the inhibition of anterior growth by certain factors brought into play through the invoking of posterior section. With these two separate factors operating on the symmetry of the head, the problem arises as to whether or not the asymmetry of the head developing from an oblique-anterior cut surface could be antagonized or reversed by imposing a posterior cut surface behind its anterior, more active region.

## MATERIAL AND METHODS

In these studies 14–16-mm. *Euplanaria dorotocephala* were used exclusively. They were collected in fresh-water springs near Chicago several weeks before being used in the experiments. They were not fed for at least 1 week before the experiments were begun. In the laboratory the water on the worms was changed on alternate days.

The test and control lots were always selected from the same stock and cared for identically. After section the animals were kept in finger bowls (25–30 per bowl) in well water until the proper stage for observation was reached. Observations were usually made with a binocular microscope but were often checked, particularly in the early stages, by means of a compound microscope.

The worms were sectioned with a very sharp straight-edged scalpel in shallow water on a glass surface. All cuts were made with a single movement of the knife to avoid jagged edges to the wound. An attempt was always made to make the cuts while the animal was fully extended.

## EXPERIMENTAL DATA

1. *The effects of oblique-posterior half-section on head formation at an oblique-anterior cut surface.*—Three experiments, involving 105 animals, were performed in which an oblique-anterior section was made at an angle of 60° from the transverse axis of the body—the left side being always the more anterior (Fig. 1).[3] It was found that

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[3] Oblique sections made at an angle of 45° from the transverse axis failed to give definite results as regards asymmetry.

section could be made with greater accuracy if the head was first removed by a transverse cut directly behind the auricles. These cases of single oblique section are called "controls." Equal numbers of animals of the same size and from the same stock were sectioned obliqely in a manner identical with that of the controls. In addition a second oblique section was made at a distance of approximately 1 mm. posterior to the first and running parallel to the more anterior

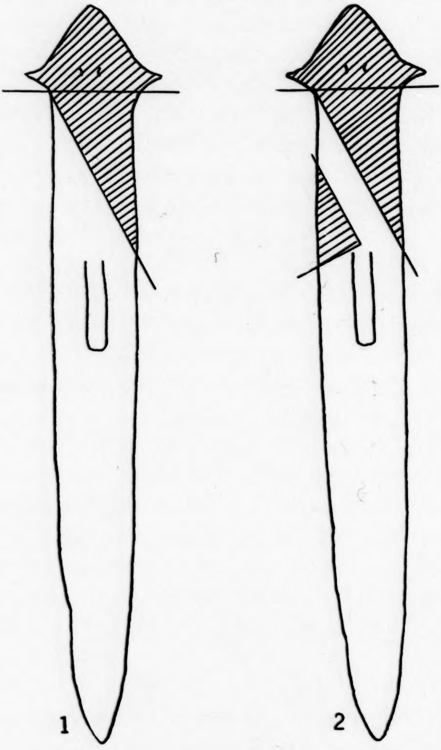

Fig. 1.—Control animals with an anterior-oblique section made at an angle of approximately 60° from the transverse axis of the body.

FIG. 2.—Test animals with a block of tissue removed posterior to the anterior region of the anterior-oblique cut surface.

region to the mid-line of the body. A third section was made at right angles to the second in such a way that a triangular block of tissue was removed from the left side of the animal and a posterior cut surface was created behind the anterior half of the oblique-anterior cut surface (Fig. 2). These animals are referred to as "tests."

The controls and tests were examined 5–6 days after the operations. The symmetry or asymmetry of the head, as well as any other developmental irregularities, was recorded. The animals were then returned to the finger bowls and observed at a later period (12–13 days after section). In Table I are recorded the data for the tests and controls at the different times of observation. As the eyes have been selected as an index to symmetry, the data are presented in comparative size of eyespots.

TABLE I

THE EFFECT OF OBLIQUE-POSTERIOR HALF-SECTION ON THE SYMMETRY OF THE HEAD APPEARING ON AN OBLIQUE-ANTERIOR CUT SURFACE

| | 5–6 Days after Section | | 12–13 Days after Section | |
|---|---|---|---|---|
| | Control | Test | Control | Test |
| Small eye, left | 8 | 21 | 21 | 24 |
| Eyes equal | 24 | 17 | 69 | 52 |
| Small eye, right | 70 | 24 | 6 | 13 |
| One eye | 0 | 9 | 0 | 3 |
| Three eyes | 0 | 0 | 8 | 0 |
| No eyes | 2 | 34 | 0 | 12 |

It will be noted in Table I that 5–6 days after section the controls have reconstituted 78 asymmetrical heads, and that in 70 the larger eye is on the left (more anterior) region of the head (Fig. 3). The test worms present quite a different picture, as out of 45 cases of unequal eyes 21 appeared with the larger eye on the right (Fig. 4).[4] Equally as important as the reversal in size of eyespots is the fact that 9 of the test worms show a single eye while in the controls none are in this class. Moreover, 34 of the tests at this period in reconstitution show no eyes (Fig. 5) while all but 2 of the controls have developed definite eyespots. It seems quite clear from these data that the oblique-posterior half-section is effective not only in reversing the difference in size of the eyes but in retarding the development of the head at the anterior cut surface.

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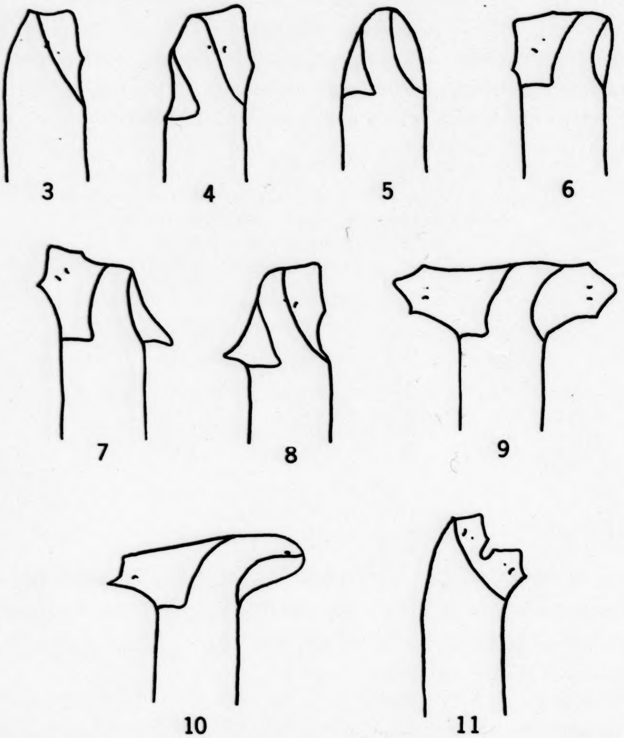
[4] The eyes of the different animals shown in the figures are not drawn exactly to scale. When speaking of a reversal in eyespot size in the early observations, it is not meant to imply that the inner eye grows faster but that the outer eye is retarded.



FIGS. 3–11.—Different forms arising in response to anterior-oblique section (with and without posterior half-section).

After the pieces have reconstituted 12–13 days (Table I) the controls have regulated considerably toward the bilateral symmetry of the species. Sixty-nine out of 104 have eyespots which are, as far as could be detected, equal. Only 6 of the 70 animals which began with the larger eye at the left have remained in this condition. Instead, of 8 individuals showing the larger eye on the right, as was the case at 5–6 days after section, 21 animals now show the larger eye on the right side. At this time 8 animals show 3 eyes, which suggests that a new eye has arisen on the right in addition to the smaller slightly misplaced eye which first appeared there.

Of the test animals after 12–13 days, 52 show eyespots approximately equal in size, compared to 17 recorded at the earlier stage of observation. Three still show a single eye, and 12 show no eyespots in the small amount of tissue at the oblique-anterior cut surface. Of these 12 with extremely inhibited anterior reconstitution, 8 closely resemble Figure 5, in which no head develops at any region. In 2 a secondary head has developed in the region where the posterior block of tissue was removed with complete inhibition of the oblique-anterior surface (Fig. 6); and in 2 a secondary head has also developed but a tail has been induced on the anterior cut surface, where a head would otherwise appear (Fig. 7).

In any work dealing with the sectioning of *Euplanaria*, forms appear which are of interest to the student of reconstitutional development. In fully one-half the test worms a tail appeared at the region where the posterior block of tissue was removed (Fig. 8). In one case (classed as "eyes equal") a head appeared on both the oblique-anterior cut surface and in the region where the block of tissue was removed (Fig. 9). It is interesting to note that in this case the inhibited eye is on the side of the secondary head which springs

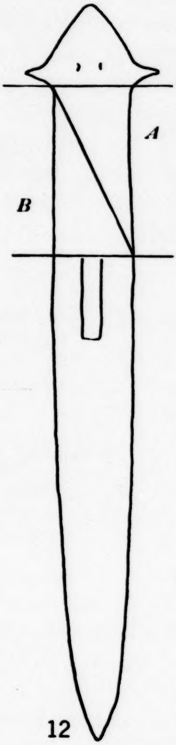


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FIG. 12.—*A*, pieces with anterior-transverse and posterior-oblique cut surfaces. *B*, pieces with anterior-oblique and posterior-transverse cut surfaces.

from the oblique-posterior cut surface. One form appeared (classed under the heading of "one eye") in which the head arising from the oblique-anterior cut surface is strongly inhibited while the secondary head is very asymmetrical with almost complete inhibition of its "anterior" side (Fig. 10). One atypical form appeared in the controls (Fig. 11) which demonstrated a mutual inhibition of the "inner" eyes when two heads arise on the same cut surface.

2. *The effect of oblique-posterior with transverse-anterior cut surfaces and oblique-anterior with transverse-posterior cut surfaces on the symmetry of the reconstituting head.*—An experiment was performed involving two lots of 25 worms each to demonstrate further the effect of the posterior cut surface in controlling the symmetry of the head. The worms were sectioned in the manner shown in Figure 12. The head and region posterior to *B* were not used in these experiments. Pieces *A* and *B*, together, make up approximately one-fourth the length of the animal. Since the results obtained from the two lots were nearly identical, they are grouped together in Table II in percentages. Shortly after section the cut ends of the pieces contract, giving the piece an asymmetrical appearance. The pieces were examined and classified on the basis of eyespot size approximately 2 weeks after section. Because of the greater area of injury there was a greater number of deaths than is usually found in reconstituting pieces of *Euplanaria*.

TABLE II

THE EFFECT OF OBLIQUE-POSTERIOR AND TRANSVERSE-ANTERIOR CUT SURFACES AND THEIR RECIPROCALS ON THE SYMMETRY OF THE NEW HEAD

| | *A* | *B* |
|---|---|---|
| Small eye, left | 58 | 2 |
| Eyes equal | 25 | 14 |
| Small eye, right | 0 | 64 |
| Teratophthalmic | 2 | 2 |
| Anophthalmic | 0 | 2 |
| Deaths | 12 | 16 |

The results are unmistakable. Not a single time does the smaller (inhibited) eye appear on the right in *A* pieces. As *A* pieces possess a transverse-anterior cut surface, it seems evident that the obliquity of the posterior cut surface is alone responsible for the asymmetry of the head developing in this case. In *B* pieces the posterior influence and the body-level (due to oblique-anterior section) unite in producing asymmetry of the head. It will be noted that only 14 per cent developed with equal eyes and that only 2 per cent showed reversal. Comparison of these data with an oblique-anterior cut surface alone (Table I) show the importance of the posterior factor. With an oblique-anterior cut surface alone, a regulation takes place early, so that within 2 weeks after section the asymmetry has disappeared or been reversed.

## DISCUSSION

The gradient hypothesis seems at present to provide the most satisfactory basis for the interpretation of these experiments on asymmetry of the reconstituting head in *Euplanaria*. In the first place, heads developing from an oblique-anterior cut surface show asymmetry in that the more anterior region of the cut surface develops an eyespot first. This fact points to an anteroposterior differential in activity within the anterior zooid. Watanabe (1935*a*) has shown quite clearly that there actually is a gradient in rate of eyespot formation in heads developing from transverse cuts at different levels along the main axis of the animal. Oblique-anterior cuts of the order used in the experiments give an anterior cut surface which is graded in eye-forming rate throughout its expanse. It is therefore to be expected that the most anterior part of an oblique-anterior cut surface should give rise to its portion of a head before the less active more posterior part.

From the onset of reconstitution of the head from an oblique-anterior cut surface there is, however, a gradual regulation toward the bilaterality of the species. This is shown rather diagrammatically in Figure 3. The part of the head arising from the most anterior region of the anterior cut surface is, in reality, made over from old tissue. The auricle and eyespot usually appear in the old tissue. In these experiments the right (more posterior) region of the head is made up entirely of new unpigmented tissue. The later growth of this new tissue is more rapid on the right than on the left sides of the head, and symmetry usually results.

With the removal of a block of tissue posterior to the anterior half of the oblique cut surface a new set of factors is imposed upon the reconstituting head. These factors tend to inhibit the side of the head which normally would have developed first. Child and Watanabe (1935) and Watanabe (1935*b*) have presented data which indicate that the factors arising at the posterior cut surface which prevent the dedifferentiation and activation of the head-forming cells at the anterior cut surface are transmitted from their posterior point of origin chiefly by way of the ventral nerve cords. In these experiments with an oblique-posterior half-section only one of the nerve cords was severed, although it is possible that occasionally the other nerve was injured because of the proximity of the cut.

A comparison of the data furnished by these experiments on the removal of a block of tissue from behind an oblique-anterior cut surface with the data presented by Child and Watanabe (1935) on the removal of a block of tissue posterior to a transverse cut surface is extremely interesting. These authors report that only 2 per cent of the reconstituting animals develop heads of a strongly inhibited type (1 teratophthalmic, and 1 teratophthalmic or slightly teratomorphic), although a large majority of the early heads are asymmetrical. The data with posterior-oblique half-section (Table I) show that 5–6 days after section (also the time of observation used by Child and Watanabe) 9 from a total of 105 animals have heads with only one eye and 34 have developed no eyes at all in the tissue of the anterior cut surface. By 12–13 days after section, 3 are still with a single eye while 12 of the 34 with strongly inhibited anterior reconstitution have failed completely to develop heads. An explanation for this difference in inhibition of heads when a posterior block of tissue has been removed is to be found in the gradient hypothesis. In the transverse sections the head arises in the middle of the anterior cut surface in response to the mediolateral gradient. The imposed posterior half-section is therefore effective in inhibiting only one side of the head, which results in its asymmetrical development but not complete inhibition. In oblique sections the situation is somewhat different, for here the head arises chiefly in response to an anteroposterior gradient and is therefore not in the middle, but on the more anterior region, of an oblique-anterior cut surface. A parallel oblique half-section imposed behind the anterior region of an oblique-anterior cut surface may therefore affect the entire head, at least in its earlier development. This results in a greater inhibition of heads arising from an oblique cut surface with posterior half-section than from a transverse cut surface with posterior half-section.

When a block of tissue is removed in order to impose a posterior cut surface upon either an anterior-oblique or an anterior-transverse cut surface, the possibility that the tissue removal itself may present factors inhibiting growth at the anterior region must not be ignored. In other words, the removal of a block of tissue posterior to the site of the new head may cause a deficiency of material which conceivably could inhibit that side of the new head behind which the tissue was removed.

That nervous factors do play a very important part in the inhibition of heads in *Euplanaria* seems to be fairly certain from the data at hand; yet there can be no doubt that tissue relationships (i.e., size of piece) will determine the ultimate size of the eyes when they do appear. It must be remembered, however, that the eyes examined 5–6 days after section have by no means attained their ultimate size. Such early inhibition does not seem likely to be due to lack of tissue, as we know from a multitude of experiments that there is sufficient material to permit the growth of much larger eyespots than were examined. The complete inhibition of eyespots (at 5–6 days) in 34 out of 105 cases with the posterior block of tissue removed points to a nervous effect rather than a tissue deficiency. The complete inhibition of one eye in 9 cases points to similar conclusions.

The appearance of a secondary head in the region where the block of tissue was removed creates an interesting problem (Figs. 6, 7, 9, 10). These heads have arisen partly from a posterior cut surface and partly from an anterior cut surface. It is interesting to note that the side of the secondary head which does arise on the posterior cut surface commonly (though not always) shows signs of inhibition of the eye and auricle (Figs. 6, 9, 10). The inhibited side of the head arises from a region which must first undergo a reversal in polarity, a factor which may produce a delay in the side of the head it affects. Tissue deficiency may also in this case be a cause of inhibition.

It has been noted in the data (Table I), and particularly in the controls, that slight indications of a reversal of size of eyespots appear at 12–13 days after section. In 8 cases it was also noted that a third eye had appeared in the regulating head. The increase in size of the eyespot arising in the new tissue over its fellow suggests that the first eyespot to appear is related to only a rudiment of the cephalic ganglion and is not at its final location. With the growth of the cephalic ganglion another eyespot locus may be determined. The enlargement and changes which occur perhaps determine either a larger or an additional eyespot field.

The data presented in this paper suggest that the work of Rand and Boyden (1913) on oblique sections may be interpreted in terms of a physiological differential along the anteroposterior axis. The reversal in eyespot size they describe when a longitudinal strip is removed from the outer side of the body suggests strongly that this operation injured to some extent the nerve cord on that side of the body and thereby produced an inhibition of the head-forming cells on the order of our experiments with the removal of a posterior block of tissue.

It has often been noted that oblique strips give rise to individuals which are much more asymmetrical in reconstitution than those which reconstitute when only a single oblique cut has been made. In the oblique strips the head appears on the extreme anterior region of the anterior cut surface, while the tail is confined to the more posterior area of the posterior cut surface. Both develop very asymmetrically. It seems to be clear that the reason for this pronounced asymmetry is because of two factors: (1) the anteroposterior differential in rate of head formation, and (2) the inhibitory influence of posterior section. In the oblique strips it appears that the only region of the anterior cut surface which can develop a head is confined to the most anterior part of the cut surface, which, presumably because of its higher physiological activity, is able to overcome the inhibitory influences which have been imposed by a parallel posterior cut surface. In this connection it may be mentioned that experiments by many students in Professor Child's laboratory have shown that very short pieces with parallel anterior and posterior-oblique cut surfaces may yield headless individuals. In this case it is apparent that the posterior cut surface has completely inhibited the entire area of the anterior cut surface. The work on oblique strips also shows quite clearly that the head does not arise in response to a stimulation by the severed ventral nerve cords. If the nerve cords were the chief factors in the stimulation, the new head would surely arise near the middle of an oblique-anterior cut surface instead of at the more anterior region.

## SUMMARY

1. A new head arising at an anterior-oblique cut surface made at an angle of 60° from the transverse axis of the body in the anterior zooid of *Euplanaria dorotocephala* is asymmetrical in that the eyespot nearest the longer margin of the piece usually develops first.

2. When a parallel oblique-posterior half-section is made behind the more anterior region of the oblique-anterior cut surface, there is a tendency for the size of the eyespots on the new head to be reversed or for the new head to be partially or completely inhibited.

3. Regulation of asymmetrical heads at an anterior-oblique cut surface takes place very early in the reconstitutional process, and the normal bilateral symmetry of the species usually results.

4. Factors which may influence the symmetry of the developing head are: (1) the anteroposterior physiological differential and (2) factors originating at the posterior cut surface which inhibit the activation and dedifferentiation of the head-forming cells.

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# DEVELOPMENTAL ANALYSIS IN PLUMAGE. I. THE INDIVIDUAL FEATHER: METHODS

(Two plates and six figures)

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## I. INTRODUCTION

INTRINSIC properties of the individual feather (capacity for regeneration, mode of growth and differentiation, and reaction characteristics) make it a valuable instrument in the analysis of certain biological problems. The "field" characteristics of the plumage tracts are not less significant.

In the series of papers in this number of *Physiological Zoölogy* we extend analysis with particular reference to the relation of measurable characteristics in the feather to the sequence of embryonic events in the regenerating germ. Several phases of this analysis have appeared in preliminary accounts (Juhn and Fraps, 1934, I–IV).

In this first paper of the group we describe methods for preparation of material, apparatus and methods for measurement of pattern components in the definitive feather, and geometric procedures based upon the application of simple constructions to photographs of mounted feathers. The geometric methods and their significance rest upon relations described in the second paper.

The second paper ("II. Plumage Configurations and the Mechanism of Feather Development") describes certain pattern configurations and the mechanism of growth in the germ which we believe required to account for the properties of these configurations.

The establishment of these relations permits the definition of gradient and symmetry relations in the plumage tracts; each tract is treated as a field, and each follicle as a point therein. These points are characterized numerically in terms of absolute magnitudes (lengths, growth-rates, etc.) and by developmental ratios.

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[1] This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research; one of the authors (R. M. F.) was the recipient of a grant-in-aid from the National Research Council.

In the third paper ("III. Field Functions in the Breast Tracts") we apply the methods described here and other measures to analysis of asymmetry and gradient relations as these are illustrated on transverse co-ordinates of the breast tracts.

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The geometric procedures described in this paper make possible the formulation of relations (e.g., barb frequencies) which are of considerable interest as phases of reaction thresholds in the different plumage tracts (see particularly Juhn and Gustavson, 1930). We shall present elsewhere the results of analyses of regenerations under the action of female hormone and thyroxin.

It need scarcely be emphasized that the phases of development recorded as measurable magnitudes or "frequencies" in the feather will often require transformation in terms of dynamic properties of the regenerating germ, e.g., growth-rates. This combination of physiological and geometric analysis is of particular value in defining the genetic complements of plumage, especially in connection with the field characteristics of the different plumage areas.

We have some data in this connection on hybrids of Barred Rock–Brown Leghorn crosses. These are of particular interest since the expression of the genic complement rests with some developmental property of the individual follicle which changes in intensity during the regenerative process and in successive regenerations (Juhn, 1933; see also Danforth, 1935).

## II. Preparation of Material

We consider here only magnitudes or frequencies which can be determined in the plane of the feather vane. These include the distribution of barbs on the shaft, length of barbs, and segments of barb and shaft defined by pigmentation or other configurations.

The most convenient orientation of the feather is with shaft straight, barbs at right angles to shaft or line of barb-shaft union. Feathers so mounted represent a system of natural co-ordinates in which shaft lengths are treated as abscissas, the barbs as ordinates. Thus, if Plate I is viewed with the shaft horizontal, apex of the feather to the left, the right vane-half is seen as a simple co-ordinate system. The left and right vane-halves of the feather shown in Plate

I are represented in text Figure 1 by lines drawn through the apexes of barbs.

A simple mounting-frame, shown in text Figure 2, is used in preparing feathers. The device consists of a board, *A*, with side members, *B* and *C*, serving as guides for the sliding member, *E*. At right angles to the line of motion of the board, *E*, is a guide bar, *D*, against which the brush or needle used in final alignment of the barbs is moved at right angles to the shaft. The feather is applied to the surface of heavy illustration board or similar material; this board, or base, is held on the sliding member, *E*, with thumbtacks during the mounting operation. Feathers with large and stiff shafts are mounted on the type of base shown in text Figure 2. This base is made up of a lower block of illustration board, *F*, upon which are cemented two upper blocks, *G* and *H*. These blocks are in contact at or near one end; at the other they are separated sufficiently to accommodate the increased width of shaft from apex to base. For feathers with relatively slight shafts—e.g., saddle and breast feathers—the groove, *K–L*, is cut or impressed into a single piece of board.

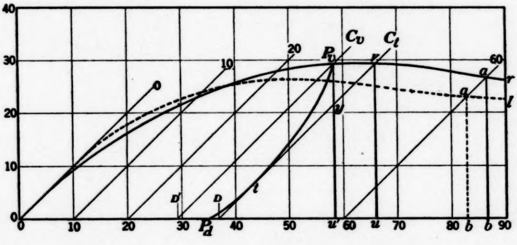

FIG. 1.—Marginal contours of the vane-halves of secondary major covert from a Brown Leghorn capon. Barbs (as *ab*, left and right vane-halves) lie at right angles to the line of barb-shaft union; the heavy curves pass through the apexes of barbs thus ordered. Abscissas represent millimeters from apex of the primary barbs; ordinates, millimeters from line of barb-shaft union. The diagonals, 0, 10, 20, and 60 represent loci of simultaneously uniform growth in the feather vane-halves; with barbs mounted at 90° to the line of barb-shaft union, the c-isochrone forms an angle of 45° with the same base line (axis of abscissas).

The curved line, $P_vP_d$, in left vane-half only, is an assumed locus of simultaneous pigmentation; maximum differentials involved in pigmentation are measured by the difference between c-isochrones $C_v$ and $C_t$ at their shaft intercepts.

In mounting the feather, the shaft is first set with hot paraffin into the groove, *K–L*. No particular attempt is made to straighten the barbs in this operation; the shaft is simply pressed into the groove and held down until the paraffin, applied with a brush, has cooled and hardened. The barbs are then pulled out at approximately right angles to the shaft with a brush carrying hot paraffin; we begin at the base and work to the apex of the feather. In the final operation the barbs are brushed out as accurately as possible into the right-angle position, and surplus paraffin is removed at the same time. The bar, *D*, serves in this operation to guide the brush as the feather is moved from base to apex under it.

A much more delicate final procedure is to bring the barbs to right angles with the shaft (or line of barb-shaft union) with a needle intermittently heated just sufficiently to melt the paraffin surrounding the barb and allow its alignment. The relatively small amount of heat carried by the needle is quickly dissipated, and the barb is "frozen" into position almost at once. However time-consuming, this technique gives us highly accurate mounts; these are particularly valuable for analysis of configurations directly from photographs. The feather represented in Plate I is an example of this technique.[2]

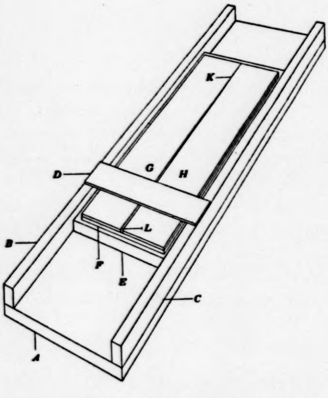

FIG. 2.—Mounting-frame used in preparation of feathers with barbs at right angles to the shaft; description in text.

Feathers thus mounted in paraffin are easily damaged; they can be given considerable protection by application of a film of shellac, collodion, or Duco. The protective film is applied with a spray.

In the permanent mounts the shaft of the feather has, of course, been straightened. Curvatures of shaft are very probably of importance, both as measures of differential rates (e.g., growth-rates) and as corrections to be applied to other data, as, for example, the exact angular relation of fault bars and shaft. We determine the curvature of shaft before mounting the feather by adjusting a flexible curve to the shaft in the required planes, and transfer these curves to paper.

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[2] We wish to express here our appreciation of the accuracy displayed in the feather mounts prepared by Miss Annelise Gerloff, some of which are reproduced in the three papers of this series.

Barbs are generally mounted at right angles to the *axis* of the shaft; in some feathers (e.g., the primaries) the shaft increases rapidly in diameter near the base. If direct measurements are made of barb and shaft lengths, no error is introduced, inasmuch as our base line is the junction of barb and shaft; in photographic analysis this actual base line—not the central axis of the shaft—must be determined and the degree of error so introduced taken into account unless barbs are mounted at right angles with it.

## III. MECHANICAL MEASUREMENT OF MAGNITUDES IN THE FEATHER

The significant configurations in the definitive feather are determined in terms of lengths or distances. The magnitudes which must be measured vary from fractions of a millimeter—e.g., distances between barbs—to well over 40 mm. for total barb lengths. Also, a relatively great range of motion is required since, as a rule, we must proceed continuously from apex to base of the feather, as in measurement of barb distribution. The machine described here satisfactorily meets these requirements.

Photographs of the apparatus are shown in Plates IIA and IIB. The fixed microscope carries cross-hairs against which all settings are made. Range of motion of the stage is 220 mm., both in the line of motion of the gauges and at right angles to this axis. Measurements of stage displacement are made with two gauges, which may be used singly or in combination: a vernier scale reading to 0.02 mm. and a micrometer reading directly to 0.01 mm. The vernier operates over the full range of stage motion; the micrometer has a range of 25 mm. and is used primarily for measurement of barbule distribution on barbs, etc.

The mounted feather is fixed to the stage with thumbtacks (or with clips, not shown). The feather in the positions shown in Plates

IIA and IIB is oriented for measurement of distances along the shaft, e.g., distances between successive barbs. Exact alignment of the shaft with the axis of motion of the gauges is obtained by rotation of the stage, adjustment *T*, and transverse displacement of the stage, adjustment *P*.

If the micrometer is locked (*M*, Pl. IIB), any point on the shaft of the feather is brought under the cross-hairs by turning the vernier adjustment, *K*. Similarly, with the vernier locked on its scale (*V*, Pl. IIB), the stage is moved by turning *L* and reading positions on the micrometer scale.

The range of the micrometer may be extended over the full length of stage motion by resetting to zero. The vernier is used to shift the stage to the end-point of the previous series of micrometer readings. Again, the vernier may be used to move the shaft to any selected point in its length, and the distance between several barbs on either side of the chosen points of reference measured off with the micrometer. This procedure is convenient and rapid for determination of barb distribution.

Measurements along the axes of barbs are made with the stage at right angles to the position just described, i.e., with the barb axis in the line of motion of the gauges.

The transverse adjustment, *P*, is for exact location of an axis. It is not sufficiently rapid for many purposes, as, for example, in the location of barbs at intervals along the shaft in order to determine the approximate contour of the feather. The required rapid shift in the transverse direction is accomplished by lifting the lever, *S*, moving the stage to approximately the point desired, dropping the lever, and making the exact adjustment with *P*.

Many pattern configurations—e.g., the marginal contour just mentioned—may be located by still another procedure. The position-scale, *u*, calibrated in 0.5 mm., is parallel to, but independent of, the line of motion in the transverse sense. A pointer, *v*, moves with the transverse carriage. With the shaft of the feather in the line of transverse motion, points along its length are set off against the linear scale. The mean barb length corresponding to these positions on the shaft is then determined in the usual manner.

The apparatus described here has proved particularly convenient in virtue of its range of stage motions, rapidity of operation, and the fact that readings are made directly from scales. Inasmuch as it may be useful in other applications, a brief note on its construction is appended (p. 316).

## IV. GEOMETRIC ANALYSIS OF REGENERATED FEATHERS

We define the collar-isochrone,[3] or "c-isochrone," as the locus of simultaneously uniform cell division in axial sense. The barb-shaft co-ordinates of the c-isochrone are constant in the following terms: *The locus of simultaneously uniform cell division in axial sense is defined in the regenerated feather by the locus of points equally distant on barbs and the shaft from points of union of barbs with the shaft.* Let $P_v u'$, text Figure 1, be any barb crossed by the c-isochrone $VD$; the c-isochrone is completely defined by the relation $Du' = u'y$. If both vane-halves are plotted with barbs at right angles to the line of barb-shaft union (abscissa of text Fig. 1), $D$ is the common point of incidence of opposed c-isochrones and the angle $VDu' = 45°$.

In terms of growth, the c-isochrone represents a locus of points surrounding the base of the germ in the region of primary cell division. The projection of the originally annular locus of cell division in the germ upon corresponding elements in the regenerated feather takes the form of diagonals (at 45° to the shaft if barbs are mounted at 90° to the shaft), in consequence of uniform axial increments in barbs *and shaft;* the important c-isochrone constant is the identity of barb and shaft co-ordinates with respect to the point of union of any barb with the shaft, and not a given angular formation.

We are concerned here solely with magnitudes and relations which can be defined on the assumption that the c-isochrone represents in fact the locus of simultaneously equivalent growth by cell division at the base of the regenerating germ. The geometric procedures are straightforward; the exact interpretation of data thus obtained must always return, however, to actual rather than inferred developmental and growth relations in the germ. We call attention to the following points particularly (these are dealt with in the second paper of this series and are noted only in summary here):

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[3] The term "isochrone" is due to Hardesty (1933), who used it to denote the locus of simultaneous pigmentation; for further description and distinctions, see Fraps and Juhn (1936*a*).

1. We assume here that the c-isochrone is defined by exactly equal barb and shaft co-ordinates. On theoretical grounds it is probable that small but highly significant growth differentials (by cell division) characterize the locus of the c-isochrone in the germ. The exact co-ordinates of the c-isochrone in the regenerated feather may thus be different (however slightly) from the ideal construction here employed.

2. All levels represented by points of incidence of the c-isochrone on barbs and shaft are assumed to represent also simultaneously equivalent loci of cell division in the germ. It does not follow, however, that a c-isochrone (or c-isochrone parallel) in the collar defines the definitive structures (barb and shaft sections) of the same locus in the regenerated feather. The c-isochrone defines *loci* of cell division, and these loci may become levels of one or another element without change in co-ordinates of the c-isochrone itself.

3. The "ideal" uniform co-ordinates of the c-isochrone are in no sense evidence that differentials in reaction do not characterize different ventro-dorsal positions in the collar. On the contrary, any simultaneously imposed reaction which is not parallel to the c-isochrone in the regenerated feather can only be understood to represent some order of differential with respect to the c-isochrone as base.

Hardesty (1933) has described methods for analysis of the regenerated guinea-fowl feather.[4] Her procedures are based on the assumption that the locus of simultaneous pigmentation in the vane is approximately parallel with the locus of growth by cell division. This is apparently true as a limiting case, but in general there is considerable difference between the two loci (Fraps and Juhn, 1936*a*). The two methods give results which differ in proportion to the differences in configurations of the base lines used by Hardesty and by ourselves.

On one point particularly, our methods and results differ from those described by Hardesty: the base line from which our analysis proceeds is defined in terms of barb and shaft co-ordinates which are applied directly to the vane of the feather. Removal of barbs and spacing them at uniform intervals, with barb apexes forming a base line, introduces curvatures and therefore differentials of variable order, where none exist according to our findings (fault bars, described in second paper of this series). The methods of Hardesty yield results at variance with ours in these instances quite apart from differences due to application of differing base isochrones.

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[4] We are, of course, concerned here with general methods. The application of relations in the regenerated feather to determination of antecedent relations in the germ is implicit in much of the analysis of Lillie and Juhn (1932), Montalenti (1934), and others. The suggestion of Lillie and Juhn that barb growth might be determined by measurement of distances between spaced female bars in male breast feathers is of particular interest in this connection.

Hardesty's pigmentation isochrone defines a perfectly tangible set of relations, although we do not believe that these relations can be referred directly to the zone of growth by cell division. The p-isochrone is particularly valuable in defining the space order of ventrodorsal differentials involved in pigmentation reactions, a point touched upon briefly below (see also Fraps and Juhn, 1936*a*).

### A. CONSTRUCTIONS ON PHOTOGRAPHS

Since the c-isochrone is a uniform construction, symmetrical in opposite vane-halves at all transverse shaft levels, any number of them may be drawn into photographs of feathers with barbs mounted at a constant angle with the (straightened) shaft. The feather reproduced in Plate I will be taken to illustrate procedures. In practice, all constructions are made with a fine stencil; relatively heavy lines and points on the photograph are required for reproduction.

Transverse shaft levels are referred to the apexes of the primary barbs as origin; shaft distances are always taken from this point as 0 unless otherwise noted. The distance from apexes of the primary barbs to the first mounted barb in each vane-half, and the spacing of the unmounted barbs in these lengths, are determined under the microscope.

Having determined the point $A$ (0 of graphs), a line is constructed from $A$ through the apexes of all barbs mounted at right angles to the shaft; this continuous line will be called the "marginal contour," and it is treated as the margin of the vane-halves without further reference to individual barb apexes.

We now lay off at right angles to the central axis of the shaft, $AB$, a series of transverse lines across the shaft. These transversals are laid off at known distances (with due reference to the usual enlargement of the feather in reproduction) from *A*, and represent *at the shaft* the axial co-ordinate of the regenerating germ to which all other data are referred. It is convenient in constructing the transversals to clamp the photograph with a straightedge parallel to the central axis of the shaft, *AB* of Plate I, and draw in the lines with a right-angle triangle.

The point at which the transversal intersects the lines of barb-shaft union in each vane-half will be called the "shaft intercept." The straightedge is next aligned with the line of barb-shaft union, *AU* of Plate I; and with a 45° triangle set at successive shaft intercepts we locate points of intersection with the marginal contour of the corresponding vane-half. These points are identified by drawing a short line across the contour at each intersection; they will be referred to as "marginal intercepts." The straight line from shaft intercept of any transversal to the corresponding contour intercept is, of course, a c-isochrone.

In general (excepting always certain apical formations) the error introduced by deviations of barbs from exact right angularity with the line of barb-shaft union is small in location of the marginal contour, relatively great in calculation of cumulative barb numbers. These latter errors are minimized by drawing from the shaft short lines lying on the perpendiculars (to line of barb-shaft union, *AU*, Pl. I) of successive marginal intercepts. In all relations involving barb numbers, the term "marginal intercept" refers to numbers *at the shaft* as determined by these perpendiculars.

We shall refer to c-isochrones by number, the number in any given case representing the distance in millimeters from apexes of the primary barbs to the shaft intercept of that c-isochrone. The o-isochrone is always the c-isochrone constructed from the apexes of the primary barbs, when these lie in the axis of the shaft. By *opposed* c-isochrones we shall denote c-isochrones in opposite vane-halves with identical shaft intercepts.

### B. MAGNITUDES AND RELATIONS DEFINED BY C-ISOCHRONES

The principal data obtained from the regenerated feather by c-isochrone constructions can best be illustrated by taking an example

(Pl. I), together with tables and graphs. All graphs presented in this connection are based upon c-isochrones at 1-mm. intervals from apexes of the primary barbs (0) to 10 mm., at 2-mm. intervals from 10 to 20 mm., and at 4-mm. intervals from 20 to 60 mm. The tables include only sufficient data to illustrate procedures.

### 1. CUMULATIVE BARB NUMBERS

The *marginal barb number*, $N_m$, is defined as the number of barb apexes lying between the apex of the primary barb (No. 1 in each vane-half) and the contour intercept of any c-isochrone. The *shaft barb number*, $N_s$, is defined as the number of barb bases lying between shaft intercepts of isochrone 0 and any c-isochrone at lower levels. Marginal or shaft barb numbers taken from the 0-isochrone through a series of c-isochrones are described as "cumulative" barb numbers.

Table I (cols. 2–5) gives cumulative barb numbers for c-isochrones located at various distances in millimeters (col. 1) from the 0-isochrone for the feather reproduced in Plate I. The complete data are given in Figure 3, curves $N_m$ representing marginal numbers, curves $N_s$ shaft numbers (ordinates), at successive axial levels (abscissas in millimeters).

Table I carries entries at 0 mm. (col. 1) for marginal barb numbers (cols. 2 and 3). These numbers represent the complement of barb loci which formed simultaneously in opposite limbs; the ventral-most barb in each collar complement defines the marginal intercept of the c-isochrone 0 (Fraps and Juhn, 1936*a*).

In all cases, marginal barb numbers represent the ventral-most limit of growth in the germ (with due regard to the notations above); and similarly, shaft barb numbers represent the dorsalmost limit of growth in the collar defined by a given c-isochrone.

### 2. BARBS ON C-ISOCHRONES

The number of barbs lying along a c-isochrone, $N_c$, is obtained by subtracting the shaft barb number from the marginal barb number defined by the same c-isochrone ($N_m - N_s = N_c$). For the c-isochrones entered into Table I (col. 1), the number of barbs on c-isochrones is given in column 6 for the left vane-half, in column 7 for the right vane-half.

Curves $N_c$ of Figure 3 represent the number of barbs on c-isochrones from apex of the primary barbs to the 60-mm. shaft level, left (*l*) and right (*r*) vane-halves. The ordinates of these curves are of course equal to the difference in ordinates of curves $N_m$ and $N_s$ (for each vane-half) at all levels from the apex of the feather (abscissas).

The barbs on a given c-isochrone are all simultaneously "represented" in the regenerating germ by cells in process of cell division.

TABLE I

PRIMARY C-ISOCHRONE DATA

| *d* | CUMULATIVE BARB NUMBERS | | | | BARBS ON ISOCHRONES | | BARB LENGTHS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Margin | | Shaft | | | | Margin | | Shaft | |
| | *l* | *r* | *l* | *r* | *l* | *r* | *l* | *r* | *l* | *r* |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) |
| 0 | 25.0 | 9.0 | 0 | 0 | 25.0 | 9.0 | 12.0 | 5.0 | ...... | ...... |
| 1 | 36.8 | 24.0 | 1.0 | 1.0 | 35.8 | 23.0 | 16.6 | 11.1 | 1.0 | 1.0 |
| 2 | 43.5 | 31.4 | 2.2 | 2.2 | 41.3 | 29.2 | 18.2 | 13.3 | 2.0 | 2.0 |
| 3 | 49.2 | 37.8 | 4.0 | 3.9 | 45.2 | 33.9 | 19.5 | 15.1 | 3.0 | 3.0 |
| 10 | 76.8 | 71.2 | 19.7 | 18.9 | 57.1 | 52.3 | 24.2 | 23.2 | 10.0 | 9.4 |
| 12 | 83.3 | 79.2 | 24.5 | 23.4 | 58.8 | 55.8 | 25.0 | 24.8 | 12.0 | 10.8 |
| 14 | 89.6 | 86.1 | 29.2 | 27.8 | 60.4 | 58.3 | 25.3 | 26.0 | 13.8 | 12.2 |
| 20 | 106.3 | 104.3 | 43.1 | 41.1 | 63.2 | 63.2 | 26.5 | 28.0 | 17.8 | 16.0 |
| 24 | 116.7 | 115.4 | 52.4 | 49.9 | 64.3 | 65.5 | 26.6 | 28.9 | 20.0 | 18.6 |
| 28 | 126.4 | 125.9 | 61.9 | 58.7 | 64.5 | 67.2 | 26.4 | 29.3 | 21.9 | 20.5 |

In terms of development, then, the number of barbs on a c-isochrone may be taken as a measure of the number of barb primordia "represented" in the collar, wholly apart from the actual number of definitive primordia present in the collar at the same moment.

### 3. BARB FREQUENCIES

The *marginal barb frequency*, $f_m$, is defined as the number of barb apexes lying between two c-isochrones 1 mm. apart at their shaft intercepts. Similarly, the *shaft barb frequency*, $f_s$, is defined as the number of barb bases lying between two c-isochrones 1 mm. apart at their shaft intercepts.

Barb frequencies based upon the data of Table I are given in Table II. Column 1 of Table II gives the *isologue* number corresponding to intervals represented between successive c-isochrones (isologue 1 is the interval between c-isochrones 0 and 1; isologue 2 is the interval between c-isochrones 1 and 2, etc.). Column 2 ($s$) records the distance in millimeters between the c-isochrones bounding the isologues of column 1.

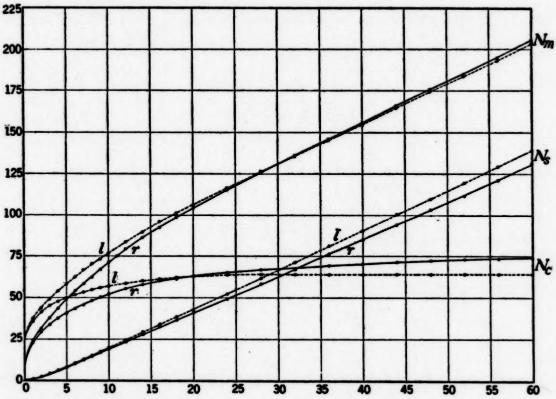

FIG. 3.—Curves representing cumulative barb numbers and number of barbs cut by successive c-isochrones, major covert No. 13, Brown Leghorn capon (Pl. I; contours shown in text Fig. 1). Abscissas, millimeters from apex of primary barbs; ordinates, barb numbers. Broken lines ($l$), left vane-half; solid lines ($r$), right vane-half. Curves $N_m$, cumulative barb numbers at the margin; $N_s$, cumulative barb numbers at the shaft; $N_c$, numbers of barbs cut by successive c-isochrones.

If c-isochrones are spaced 1 mm. apart, the marginal barb frequency is obtained directly by subtracting the marginal barb number defined by a given c-isochrone from the marginal barb number of the succeeding c-isochrone (col. 2 of Table I, $36.8-25.0=11.8$; entered under col. 5 of Table II). Shaft barb frequencies are obtained similarly from shaft barb numbers (cols. 9 and 10, Table II).

If c-isochrones are spaced at intervals other than 1 mm., marginal barb frequencies are calculated according to the relation

$$f_m=\frac{n_m}{s},$$

where $n_m$ is the number of barb apexes between successive c-isochrones and $s$ is the distance in millimeters between successive c-isochrones at their shaft intercepts. Shaft barb frequencies are calculated similarly, except that $n_s$, the number of barb bases between successive c-isochrones, replaces $n_m$, and we have

$$f_s = \frac{n_s}{s} .$$

Barb frequency curves for the secondary covert reproduced in Plate I are shown in text Figure 4 ($f_m$, marginal barb frequencies; $f_s$, shaft barb frequencies; $l$ and $r$, left and right vane-halves; abscissas, distances from c-isochrone 0). The curves are based upon the smooth curves, $N_m$ and $N_s$, of text Figure 3. This procedure tends to eliminate fortuitous fluctuations and to reduce errors in the original measurements, but obviously must be used with due regard for the nature of initial data. The circles and disks of text Figure 3 represent actual determinations.

TABLE II

BARB FREQUENCIES

| | | $n_m$ | | $f_m$ | | $n_s$ | | $f_s$ | |
|---|---|---|---|---|---|---|---|---|---|
| $d$ | $s$ | $l$ | $r$ | $l$ | $r$ | $l$ | $r$ | $l$ | $r$ |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |
| 1...... | 1 | ...... | ...... | 11.80 | 15.00 | ...... | ...... | 1.00 | 1.00 |
| 2...... | 1 | ...... | ...... | 6.70 | 7.40 | ...... | ...... | 1.20 | 1.20 |
| 3...... | 1 | ...... | ...... | 5.70 | 6.40 | ...... | ...... | 1.80 | 1.70 |
| 12...... | 2 | 6.5 | 8.0 | 3.25 | 4.0 | 4.8 | 4.5 | 2.40 | 2.25 |
| 14...... | 2 | 6.3 | 6.9 | 3.15 | 3.45 | 4.7 | 4.4 | 2.35 | 2.20 |
| 24...... | 4 | 10.4 | 11.1 | 2.60 | 2.78 | 9.3 | 8.8 | 2.33 | 2.2 |
| 28...... | 4 | 9.7 | 10.5 | 2.43 | 2.63 | 9.5 | 8.8 | 2.38 | 2.2 |

### 4. RATE FUNCTIONS OF C-ISOCHRONES

Barb frequencies represent the actual numbers of "discrete events" which, *after the completion of regeneration*, have been effected per millimeter axial growth. Within the limitations of this definition, we may speak also of "rate functions," marginal and shaft. In order not to confuse collar level and time of origin of the definitive barb primordium with c-isochrone determinations, we shall define the *marginal rate function* as the number of barb apexes determined per day *as ventral loci* with respect to c-isochrone constructions. The shaft rate function is correspondingly the rate at which the loci of union of barb bases with shaft are determined by c-isochrones.

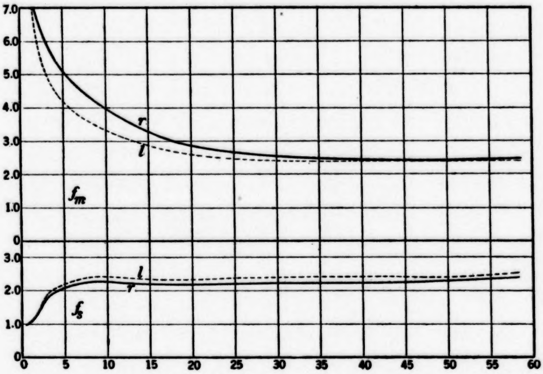

FIG. 4.—Marginal and shaft barb frequencies, major covert No. 13, Brown Leghorn capon; broken lines ($l$), left vane-half; solid lines ($r$), right vane-half. Abscissas, distances in millimeters from apexes of primary barbs; ordinates, number of barbs per millimeter at the margin ($f_m$) and at the shaft ($f_s$). Based upon the smooth curves of text Figure 3.

Knowing the axial growth of the germ,[5] $g$, in millimeters per day, the marginal rate function, $r_m$, is obtained from the marginal barb frequency according to the relation

$$r_m = g f_m \, .$$

The shaft rate function, $r_s$, is obtained similarly, except that the shaft barb frequency replaces the marginal barb frequency in the foregoing relation, and

$$r_s = g f_s \, .$$

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[5] We refer to longitudinal growth of the germ, which, of course, is continuous for the shaft during the course of a regeneration, as the axiai growth or (growth-rate) of the germ; tangential growth (or growth-rate), effecting the ventrodorsal motion of barb primordia, does not enter directly into the relations under discussion here (see Fraps and Juhn, 1936*a*).

In the limited but exact terms here defined, the rate functions of c-isochrones are a measure of one phase of differentiation in the regenerating germ; the same is, of course, true of barb frequencies. The subject requires further examination in terms particularly of the relation between axial and tangential growth motions (Fraps and Juhn, 1936*a*).

### 5. GROWTH-RATES IN INDIVIDUAL BARBS

The lengths of all barb segments lying between two c-isochrones are equal among themselves, and they are equal also to the length of shaft lying between the same c-isochrones. On the grounds of the usual assumptions concerning growth, we interpret this to mean that the rate of axial growth by cell division is simultaneously uniform in a basal annular locus of cell division (Fraps and Juhn, 1936*a*). Stated differently, points of c-isochrone incidence on the shaft and all barbs are levels of simultaneously uniform axial growth-rate.

The rate of growth of the shaft can be measured during the course of regeneration. These measurements involve growth by cell division and growth due to increase in size of cells following cell division. It is necessary to consider this second phase of growth a constant function (in total increment effected, not in rate) of growth by cell division, whence it follows that axial growth by cell division within the collar (uniform on a given c-isochrone), remains a constant function of the measured total growth of the shaft.

### 6. TERM OF BARB GROWTH

If the growth curve is known for a regenerated feather, the term of barb growth is determined by locating c-isochrones through the apex and base of the individual barb; the term of growth is identical with the time required for growth of the shaft segment so defined.

If the axial rate of growth of the shaft can be taken as constant through the term of a barb's growth, the term of barb growth is obtained by the relation

$$t=\frac{l}{g},$$

where $t$ is the time in days required for growth of the barb, $l$ is the length of the barb in millimeters, and $g$ is the axial growth of the shaft in millimeters per day through the interval of barb growth.

### 7. BARB LENGTHS

Curves for barb lengths at completion of barb growth, barbs ranged at right angles with the shaft, are identical with marginal contours as these have been defined previously (text Fig. 1: ordinates, barb lengths in millimeters; abscissas, distances on shaft in millimeters).

Since the c-isochrone is symmetrical at shaft intercepts, barb lengths in opposite vane-halves at any c-isochrone shaft intercept represent lengths of barbs simultaneously completed. If these opposed barbs are equal in length, their apical (marginal) c-isochrone loci were also simultaneously determined; if different in length, the difference can be reduced to a time interval separating origin of the marginal loci in opposite vane-halves.

The marginal intercepts of opposed c-isochrones represent loci of simultaneous origin (apexes *a*, right and left vane-halves, c-isochrone 60, text Fig. 1). The distances of these points from the shaft (ordinates) represent therefore the lengths attained by simultaneously defined barb apexes at completion of barb growth. The complete curves for axial displacement of marginal loci of simultaneous determination are given in text Figure 5 for the same feather represented in text Figure 1 (see also Table I). Note particularly the striking differentials between opposite vane-halves through first 10 mm. of feather growth.

An obvious significance of barb lengths in either of these instances is that they represent time intervals of axial growth of the individual barb in the germ (see secs. 5 and 6 above); other relations into which we cannot enter here involve rates of tangential displacement of the defined loci.

### 8. GEOMETRIC VARIABLES IN PIGMENTATION FORMATIONS

Pigmentation formations in the germ may be effected simultaneously at all ventro-dorsal positions in the collar (Lillie and Juhn, 1932; Hardesty, 1933). We shall refer to such simultaneous formations as *p-isochrones*. We consider here only relations which are defined by application of the c-isochrone construction to these simultaneously determined configurations.

Let $P_vP_d$, text Figure 1, represent a p-isochrone in the left vane-half of the feather. If any p-isochrone is not parallel to the c-isochrone, c-isochrones constructed through various loci of the p-isochrone will have different shaft intercepts. Thus, in text Figure 1, a c-isochrone drawn through $P_v$ (ventral-most limit of pigmentation in the germ) will intersect the shaft at $D'$; similarly, a c-isochrone, $C_t$, drawn through $t$, the level of pigmentation nearest the base of the germ, will intersect the shaft at $D$. The two points, $D$ and $D'$, are separated by a distance which is equal to $P_vy$, and determine therefore the maximum differential in level of pigmentation for this particular formation. We may proceed similarly with c-isochrones through $P_d$, or with c-isochrones drawn through any selected order of loci lying along the p-isochrone.

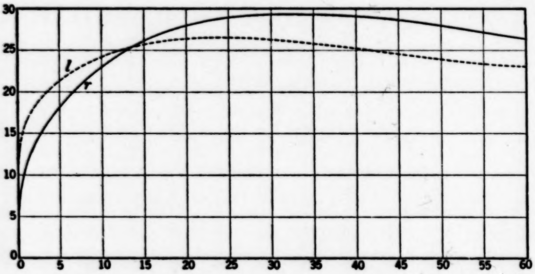

FIG. 5.—Lengths of barbs in left (*l*) and right (*r*) vane-halves at corresponding marginal intercepts of c-isochrones; major covert No. 13, Brown Leghorn capon. Abscissas, distances in millimeters from apexes of primary barbs; ordinates, barb lengths in millimeters.

The difference between cumulative barb numbers at the margin of c-isochrones $C_t$ and $C_v$ determines the least number of barb loci represented in the germ for this particular configuration (Fraps and Juhn, 1936*a*).

The significance of the measures derived by c-isochrone constructions to p-isochrone configurations is that the variable configuration of the p-isochrone can be reduced to quantitative (if relative) indexes. Several of the more general relations and applications may be touched on briefly here.

Pigmentation isochrones generally show an increasing deflection from the c-isochrone with increasing distance from the apex of the feather. This increasing deflection can be reduced to numerical relations (as the distance, $P_v y$, or differences in marginal barb numbers).

Feathers of differing types (breast, wings, etc.) show characteristic differences in deflection of p-isochrones from c-isochrones: these differences are of particular interest in connection with their dependence upon physiological properties of the regenerating germ (Lillie and Juhn, 1932).

In a perfectly symmetrical feather, p-isochrones in opposite vane-halves are symmetrical by c-isochrone measures. If the p-isochrones are asymmetrical, the degree of asymmetry under differing conditions, at different axial levels of the same feather, and in different feathers, is determined in comparable and numerical terms by c-isochrone constructions. The procedure rests upon the determination of shaft intercepts of c-isochrones through *homologous* points on the p-isochrone locus (as the point $P_v$, text Fig. 1).

If we know the form of the p-isochrone at a given level of a particular feather, pigmentation configurations departing from the p-isochrone can be reduced to at least relative measures of the time differential involved in the germinal reaction by application of c-isochrones.

## V. Relative Ratios in the Analysis of Asymmetries

We are justified in speaking of the "asymmetry" of an individual feather only with respect to exactly defined magnitudes or relations in opposite vane-halves. The feather used to illustrate c-isochrone procedures (Pl. I) is, for example, obviously asymmetrical by every measure considered in the foregoing section (text Figs. 1, 3–5). But a satisfactory statement of asymmetry requires much more than this; it requires, first, a more compact treatment of the data; and, secondly, a dissociation of "independent" and "dependent" variables entering "asymmetrically" into the developmental process.

We are concerned here, as one phase of methods, with a simple relation which describes cumulative barb numbers in opposite vane-halves of many, if not all, feathers from certain tracts. The generality of the relation and its significance in development must be the subject of further investigation.

If we let $y$ represent the marginal barb numbers on successive c-isochrones in one vane-half, $x$ the marginal numbers on corresponding c-isochrones in the opposite vane-half (curves $N_m$ of text Fig. 3), the relation between the two magnitudes is

$$y=bx+a\ ,$$

where $b$ and $a$ are constants over the range for which the relation obtains. The constant $b$ represents the ratio of cumulative barb numbers in opposite vane-halves on successive c-isochrones; if $b=1$, marginal barb numbers increase absolutely by identical increments. The value of $a$ in the equation is the value which $y$ assumes when $x$ is set equal to zero.

A similar relation is found to hold also between shaft barb numbers determined by opposed c-isochrones (curves $N_s$ of text Fig. 3 represent the initial data).

The equation $y=bx+a$ is, of course, the equation for a straight line on arithmetic co-ordinates. Wherever cumulative barb numbers (margin or shaft) are related according to this equation, numbers in one vane-half plotted against corresponding numbers in the opposite vane-half should fall along a straight line; the slope of this line is defined by $b$; and the $y$-intercept of the line is equal to $a$.

Text Figure 6 represents the cumulative barb numbers of the feather reproduced in Plate I treated as relative ratios. The relative marginal ratios (*ab* and *bc* of text Fig. 6) are based upon the actual data (not the smoothed curves) represented along curves $N_m$ of text Figure 1; the shaft ratios are based likewise on the actual c-isochrone determinations.

The relative ratio of marginal numbers falls into two well-defined phases. For the first phase, *ab* of text Figure 6, $y=1.20x-20.6$; for the second phase, *bc* of text Figure 6, $y=1.05x-7.3$. The relative rate of increase in marginal barb numbers is thus higher in the right vane-half for both phases (compare the curves of text Fig. 3).

The first plotted point, *a* of text Figure 6, represents the marginal intercepts of c-isochrone 0 (see p. 303), and is therefore referable to the number of barbs simultaneously determined in opposite vane-halves. In all cases where $b$ becomes a constant from this point, as through the phase, *ab*, text Figure 6, we may consider the simultaneously determined values of c-isochrone 0 ($y=9$, $x=25$, text Fig. 6) to be the proper origin of the curves, and the constant, *a*, then has no direct significance. The grounds for this treatment (assuming it to hold generally) are that serial formation of barb loci measured by the marginal numbers can begin only after formation of the simultaneous initial complements, and it is only these serial formations which can be treated as ratios.

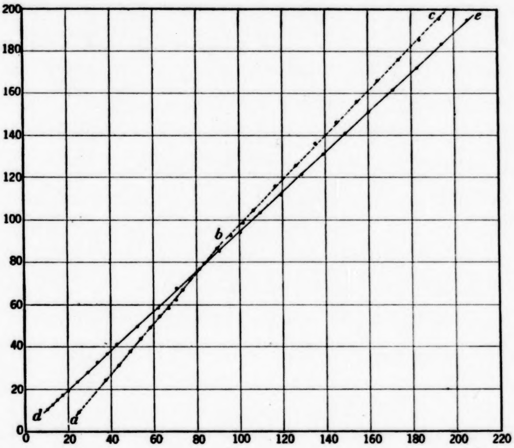

FIG. 6.—Relative ratios in increments of barb numbers in opposite vane-halves, major covert No. 13, Brown Leghorn capon (Pl. I). Abscissas, barb numbers in left vane-half; ordinates, barb numbers in right vane-half. Marginal numbers (circles) lie along the broken lines; for the first phase (*a* to *b*), $y=1.198x-20.6$; for the second phase (*b* to *c*), $y=1.053x-7.3$. Shaft numbers lie along the solid line (*d* to *e*); $y=0.94x+0.5$.

Barb numbers for shaft intercepts of successive c-isochrones are plotted also in text Figure 6 (along the solid line, *de;* *y*-co-ordinates, right side; *x*-co-ordinates, left side). Over the range shown, $y=0.94x+0.5$. Theoretically, it may be supposed that *a* should equal 0 for shaft numbers, since barb numbers at the shaft must begin with the common base of the primary barbs; this point requires further and exact analysis.

If marginal and shaft barb numbers in opposite vane-halves increase by constant ratios, it follows also that barb numbers lying along successive c-isochrones in opposite vane-halves change (increase or decrease) by a constant ratio. This relation, and others of a less obvious character, will be treated in a more extended analysis.

We have found that the relation $y=bx+a$ holds generally in describing the relative barb ratios (marginal, shaft, and "collar") in opposite vane-halves of contour feathers, secondary coverts, and possibly tail feathers of the Brown Leghorn capon. Certain primary flights are not apparently subject to the relation (nor to the heterogeny formula of Huxley, 1932). In at least one well-defined instance (hybrid: Barred Rock ♂, Brown Leghorn ♀), the genetic composition appears to impose periodic fluctuations asymmetrically in opposite vane-halves.

In certain instances where the formula holds, the values of the constant *b* for at least marginal barb numbers are definitely related with other asymmetry "indexes" (Fraps and Juhn, 1936*b*). In other instances no such general relation is apparent, as, for example, in certain coverts which are highly asymmetrical in appearance (the "appearance" is referable to marked differences in lengths of barbs) but in which the relative ratios of both margin and shaft numbers are close to unity through the main vane-structure.[6]

Relative increments in cumulative barb numbers at margins and shafts must involve, in part at least, growth vectors (Fraps and Juhn, 1936*a*). Axial growth of the germ is largely additive or "accretionary" (Huxley, 1932); and it might be supposed that, following completion of the collar complements and serial formation of barb primordia at the ventral triangle, tangential growth is to be treated also as primarily accretionary. On these grounds it might be concluded that the formula $y=bx+a$ rests in part upon a constant differential growth relation in an "accretionary system," corresponding therefore to Huxley's (1932) expression $y=bx^k+a$ in the multiplicative system. But cumulative barb numbers involve also what might be called "differentiation frequencies," and we are inclined to forego interpretation until more comprehensive results are in hand. It is also altogether possible that further analysis will show the simple relation described here to be a special and limited aspect of a more general and inclusive relation.

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[6] Although we have determined the value of *b* for a large number of individual feathers, its general significance probably cannot be known until we have much additional data on the order of change of asymmetry measures for complete series of feathers in different tracts—a point which is clearly emphasized also by those instances in which the relative ratios of barb numbers depart considerably from unity although the feathers are fairly symmetrical in appearance.

## VI. Summary

1. Methods are described for mounting individual feathers with barbs at right angles to the shaft (or line of barb-shaft union).
2. An apparatus is described for the rapid measurement of magnitudes in the vane of mounted feathers; construction of the apparatus is appended (p. 316).
3. The c-isochrone is defined in terms of growth characteristics in the regenerating germ (based upon Fraps and Juhn, 1936*a*), and its application to photographs of mounted feathers is described. Magnitudes and relations determined by the c-isochrone construction are defined, and their significance in terms of development is discussed briefly.
4. Relative ratios in barb numbers, as these are determined by the c-isochrone, are considered in connection with the formulation of asymmetries.

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## APPENDIX

## CONSTRUCTION OF THE MEASURING APPARATUS

The entire apparatus is mounted on an aluminum casting, webbed for rigidity. For linear stage motions we have made use of the carriage mechanism of computation machines. These consist of a truck, *b* (Pl. IIA), moving on ball bearings within the carriage frame, *a*. The frame of the lower carriage is bolted to the aluminum base. Its truck carries a heavy plate, *c*, to which is bolted (at right angles to *c*) a second plate, *d*; these plates are centered with respect to each other.

The upper or transverse carriage is identical with the lower; its frame is bolted to the transverse plate, *d*, and its truck carries a plate corresponding to *c* on the lower truck.

Rotation of the stage is accomplished through worm and gear. The gear, *e*, is centered in the upper truck plate, turning on a large ground bearing. A circular stage plate is bolted to the worm gear; stages proper—several types have been used—bolt directly to this plate. The worm for transmitting motion to the gear is fixed in the center of the shaft, *t*, which is supported by bearings at each end of the upper truck plate.

Change in position of the transverse carriage truck is effected by rotation of the screw, *p*, with consequent displacement of the riding half-nut, *r*. The half-nut is attached—by way of the hinged strip, *f* (Pl. IIB)—to the truck plate of the transverse carriage; it is held in position by the spring acting through the lever, *S*. If this lever is raised, the half-nut is lifted clear of the screw threads and the transverse truck may be shifted to any position.

The position-scale, *u*, is attached to the lower truck plate; the pointer, *v*, is fixed to the upper truck plate. The pointer is a strip of spring steel bearing a mark practically in the plane of the scale calibrations.

Relations of vernier scale, micrometer, and lower carriage truck are clear from Plate IIA; details are shown in Plate IIB. Axes of motion of the scales must, of course, be in exact alignment with the axis of motion of the lower truck.

The vernier scale is mounted on a pair of end-blocks above a heavy bar, *z*, which bar is bolted to the base of the apparatus. A pinion at the end of the shaft carrying *K*, engages a rack, *j*, fixed to the bar carrying the vernier scale. Pinion and shaft are held in position by a guiding frame which serves also for displacement of the vernier on its scale. The frame consists of a pair of side plates, *w* (and its opposite), spaced by the block, *x*, and bearing at their inner and lower corners the guides, *y* (and an opposite); these guide bars engage corresponding grooves in the bar, *z*.

The block, *x*, is slotted across its upper face to receive the vernier block, *i*. The vernier, then, lies with a shoulder of the spacing block on either side of it; displacement of the block brings one or the other of these shoulders against the vernier, and so moves it along the scale. The spacing-block does not touch the scale, and points of contact with the vernier are convex and in the line of the central axis of its motion. Movement of the vernier is thus free of possible distortion either of the scale itself or of alignment of the vernier with respect to the scale.

The original jaws of vernier and micrometer frame are removed except for small brackets for their union (*q*, Pl. IIB). The micrometer is of the type designed for internal measurements, in which the spindle, *m*, does not revolve with the thimble, *L*. A connecting rod, *n*, ties the micrometer spindle to the bracket, *o* (Pl. IIA), which bracket is in turn fixed with respect to the lower carriage truck.

Fairly heavy construction has been necessary throughout, largely in consequence of the ranges of stage motions required of the apparatus. There appear to be no appreciable strains on the gauges, however, nor is there any evidence of inertial drags even in rapid operation of the vernier over relatively great lengths. The micrometer has been repeatedly checked to within 0.002 mm. in return to a previously located point—about the limit of estimation possible between 0.01-mm. calibrations.

We have made considerable use of the apparatus in measurement of relations in split germ preparations, a procedure which requires illumination by transmitted light. Due to space limitations, the usual microscopic equipment is impracticable. A light source adequate to our requirements was obtained via a simple "neon type" installation (Courtney and Schopp, 1934). The discharge tube—of about ⅜-inch diameter—is bent back upon itself several times to form a series of lengths lying close together and in a plane. The unit is fitted into a shallow container which replaces the stage shown in Plate II; a ground glass plate fitting into the side walls of the container bridges the parallel sections of the illumination tube. A pair of clips hold the slide in place.

## EXPLANATION OF PLATES

All figures are photographs.

### PLATE I

Photograph of major covert over secondary No. 13 (counting from wing tip through primaries), showing c-isochrone constructions. Central axis of the shaft, *AB*; line of barb-shaft union, *AU* (in right vane-half). Marginal contours drawn only where required for c-isochrone intercepts. The herringbone diagonals, 0, 10, 20, 60, represent c-isochrones in each vane-half at levels corresponding to distances in millimeters from apex of the feather indicated by these numbers. The shaft transversals defining these distances ($D=60$ mm. from apex) are drawn at right angles to the central axis of the shaft. If barbs are mounted at right angles to line of barb-shaft union, *AU*, all other constructions assume this line as base. The angle $VDU=45°$ and locates the point *V* on the marginal contour. The point *u* is determined by the perpendicular passing through *V* from *AU* as base; similarly, the point *a* is located as the point of intersection with the marginal contour of a line from *D* at right angles to *AU*. If a number of c-isochrones are drawn into the vane of a feather, points *a* and *V* are distinguished by the angle of intersection of short lines with the marginal contours (90° at *a;* 45° at *V*); points *D* and *u* are identified by short lines of differing lengths.

### PLATE II A

Photograph of the apparatus used in the measurement of distances in feathers. The feather on the stage is oriented for measurement of distances along the shaft, e.g., distances between barbs. For measurement of barb lengths, the stage is rotated until the shaft lies at right angles to the position shown. Relatively great lengths are measured with the vernier (the micrometer is locked) by rotation of *K*. For more accurate measurements, the micrometer *L*, is used (the vernier is locked). Range of stage motions of the apparatus is 220 mm. The vernier reads to 0.02 mm.; the micrometer, directly to 0.01 mm.

### PLATE II B

Photograph showing details in combination of the vernier and micrometer scales. The micrometer is locked with the setscrew, *M;* the vernier, with the setscrew, *V*. Description in text; details of construction in Appendix.

## PLATE I

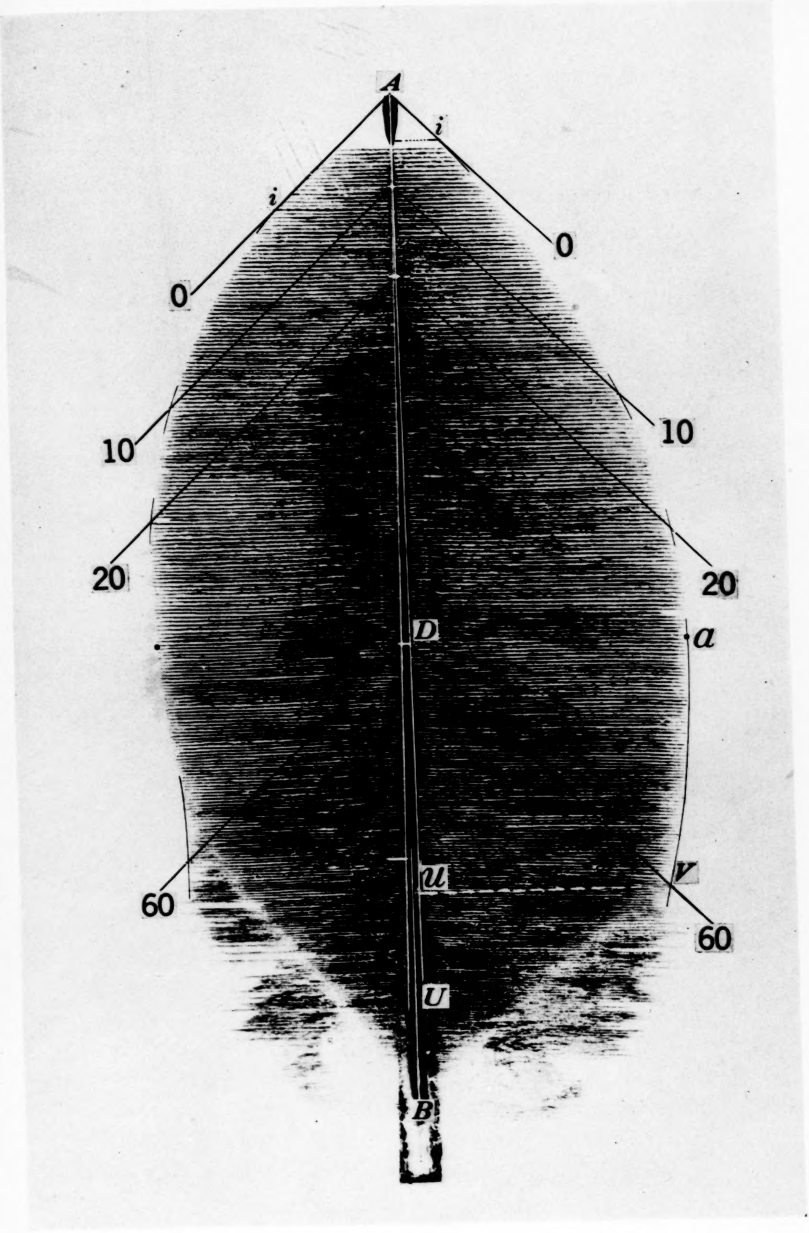

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PLATE II A

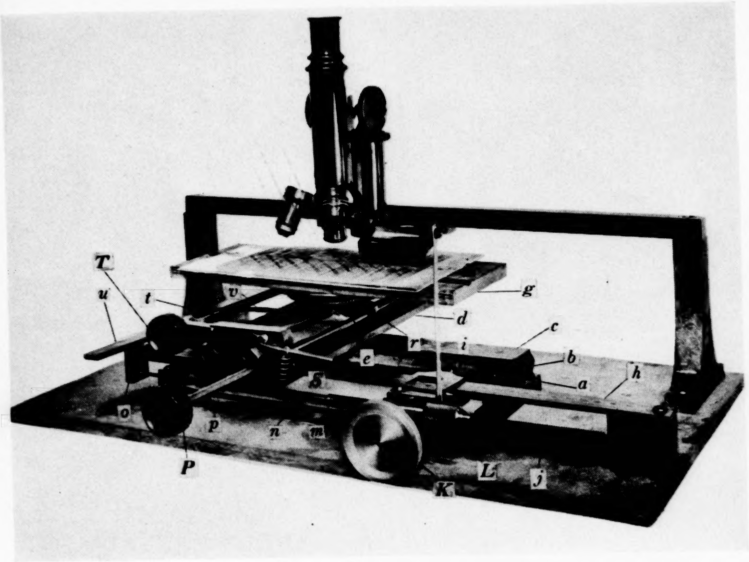
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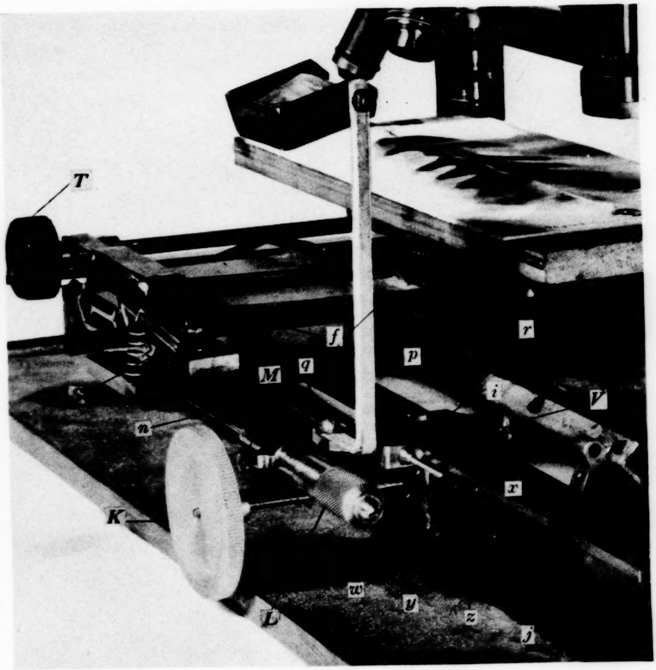
PLATE II B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# DEVELOPMENTAL ANALYSIS IN PLUMAGE. II. PLUMAGE CONFIGURATIONS AND THE MECHANISM OF FEATHER DEVELOPMENT

(Five plates and nineteen figures)

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## I. INTRODUCTION

IN THEIR analysis of growth-rate and pattern in feathers, Lillie and Juhn (1932) demonstrated that pigmentation patterns simultaneously induced in all the reactive elements of the germ come to lie, with completion of the regenerative process, in a line approximately at right angles to the shaft. Hardesty (1933) later showed that the series of simultaneously formed "spots" of the guinea-fowl feather could be treated as localized regions of reaction along lines approximately perpendicular to the shaft, although "the direction of these lines relative to the rhachis is subject to considerable variation."

On the basis of observations on the axial displacement of simultaneously induced pigmentation reactions following their appearance in the germ, Lillie and Juhn reached the conclusion that the axial rate of growth of the individual barb is highest at the apex and lowest at or near the base. The order of difference in apicobasal growth-rates was estimated to be several-fold. Hardesty obtained a similar curve for the individual barb growth-rate by analysis of pigmentation lines in completely regenerated feathers.

Juhn and Fraps (1934*a*) attempted to determine axial growth-rates of barbs by analysis of lines of simultaneous pigmentation and of fault-bar incidence (Riddle, 1908) in regenerated feathers. Taking into account results based only upon pigmentation configurations, growth-rate differentials estimated according to our procedures were

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[1] This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research; one of the authors (R. M. F.) was the recipient of a grant-in-aid from the National Research Council.

a small fraction of the differentials obtained either by Lillie and Juhn or by Hardesty.

In the present paper we give results of further analysis of configurations in the completely regenerated feather. The simple and critical relation with which we are mainly concerned is that which defines the locus of axial growth by embryonic cell division in the regenerating germ. This locus of growth will be denoted the *c-isochrone*,[2] and it is completely defined in either vane-half of the regenerated feather as follows: *Points of incidence of the c-isochrone on the shaft and any barb are equidistant from the point of union of that barb with the shaft.* The c-isochrone is symmetrical with respect to a common transverse shaft level and is uniform in all feathers. It can be located, therefore, through the entire vane of the regenerated feather if a single point of incidence is known or given.

The experimentally determined geometric relations are shown in text Figure 1, which represents a portion of completely regenerated feather, base down, barbs removed below $u$ in left and right vane-halves. Let $C_d$ be the common point of incidence of the c-isochrones, $C_vC_d$, in opposite vane-halves. Considering only the left vane-half, let $u'w = b$, the barb co-ordinate, and $u'C_d = s$, the shaft co-ordinate. The general relation is that $b = s$ for any barb between the marginal limit, $C_v$, of the c-isochrone and the central limit, $C_d$. If the barbs are ranged parallel, as in text Figure 1, the c-isochrone is a straight line, $C_vC_d$, the base of the isosceles triangle, $C_vuC_d$.

The approximate form of the lines of simultaneous pigmentation described by Lillie and Juhn and by Hardesty is indicated by $P_vC_d$. The barb and shaft co-ordinates of the pigmentation configuration, $P_vC_d$, obviously cannot be identical with the co-ordinates of the c-isochrone, $C_vC_d$. In general, $b$ ($u'w''$ of text Fig. 1) is greater than $s$ ($u'C_d$ of text Fig. 1) for pigmentation isochrones if these are considered as approximately straight lines from margin to shaft.

The c-isochrone in the vane-halves of the regenerated feather represents the locus of simultaneous reaction through the zone of

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[2] The term "isochrone" was first used by Hardesty (1933) with reference to the locus of simultaneous pigmentation in the germ. We have given the term a somewhat different meaning; the notation "c-isochrone" refers to the locus of primary cell division, the collar of Lillie and Juhn (1932, p. 139).

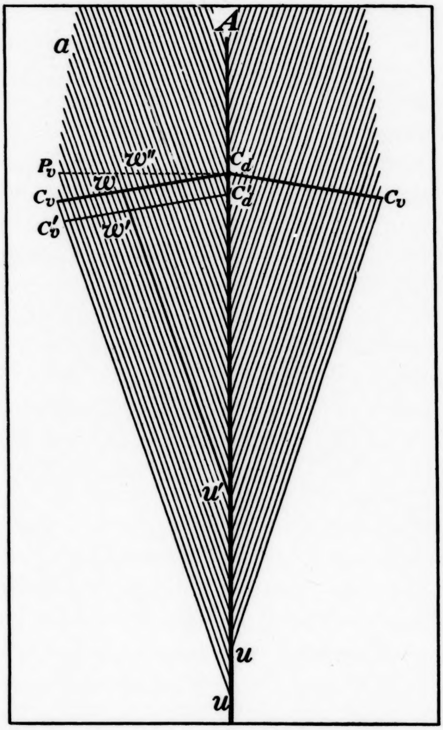

FIG. 1.—Limiting configuration of the locus of simultaneous cell division in the vane of the regenerated feather. The central heavy line represents the shaft; the barbs composing each vane-half are in approximately normal positions but are parallel with each other in each vane-half. Barbs basal to *u* are removed in each vane-half. The limiting locus of simultaneous growth, the c-isochrone, is the straight line, $C_vC_d$, across each vane-half. The barb-shaft co-ordinates of the c-isochrone are defined as the lengths of barb and shaft from point of c-isochrone incidence to point of union of the barb with the shaft. The limiting c-isochrone co-ordinates are that $C_vu$ shall equal $C_du$, $wu'$ shall equal $C_du'$, and similarly for every barb along the locus $C_vC_d$. A second c-isochrone, $C'_vC'_d$, will be parallel with $C_vC_d$, and is therefore separated from $C_vC_d$ by equal lengths on all barbs and the shaft; thus, $C_vC'_v = ww' = C_dC'_d$. Compare these relations with the germ represented in text Figure 2. $P_vC_d$ is the approximate configuration of a pigmentation boundary imposed simultaneously from ventral to dorsal limits of the germ and also simultaneously with $C_vC_d$.

growth by cell division in the germ. Since this locus of embryonic growth occurs as a straight line, with uniform co-ordinates, a second c-isochrone, $C'_vC'_d$, will be parallel to $C_vC_d$. The second c-isochrone, $C'_vC'_d$, is parallel to the base, $C_vC_d$, of the isosceles triangle, $C_vuC_d$, and must therefore intercept equal lengths on $uC_v$, $uC_d$, and all lines (barbs) parallel to $uC_v$. In terms of axial growth, successive c-isochrones intercept equal increments of growth by all barbs *and the shaft*, and these equal growth increments are laid down in the same time interval. If we accept the usual assumptions respecting growth in the germ, the axial rate of growth of all elements (barb and shaft primordia) in process of growth at any instant must be uniform.[3] This fact must be taken into account by any theory of growth relations in the developing germ.

According to Lillie and Juhn (1932), the shaft primordium of the definitive feather is a bilateral structure, the two halves of which are coextensive with the circumferential base of the germ. The definitive shaft is formed by concrescence of the bilateral primordia. The growth centers of the individual barb are assumed to be transposed from their site of origin to their site of union with the shaft proper by the ventrodorsally directed motion of the shaft primordia. The assumption of bilateral origin of the shaft accounts, therefore, for fixed barb growth centers and the transposition of these individualized centers around the base of the germ.

Considered without reference to other relations, simultaneously uniform axial increments might be accommodated to the theory of development formulated by Lillie and Juhn, although assumptions of a difficult and complex order are required. But the theory of concrescence requires also that a mathematically exact relation shall obtain between the distribution of ridges (barb primordia) in the germ and the distribution of barbs on the shaft of the regenerated feather. Analysis of this relation shows that it does not satisfy the conditions required by theory (see pp. 367–71).

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[3] We cannot be certain that growth-rate differentials of an order smaller than we have been able to detect do not characterize the collar of the germ. Growth-rate differentials, of however small an order, would be of definite significance in connection with the reaction differentials described by Lillie and Juhn (1932). We use the term "uniform growth increments" throughout this paper with this reservation in mind.

In the alternative formulation of organization and development required by our results, we assume that the shaft arises from a definitely localized region of the embryonic ring of cells at the base of the germ, and that the axial component of growth by cell division in the shaft is identical with the axial growth component of the barb complement at any instant. These relations are shown in text Figure 2, which represents one-half of a regenerating germ spread into the plane of the paper. The ventral superficial axis of the germ is to the left of the figure; the dorsal superficial axis is to the right. Ridges arise at the face of the ventral triangle, $v-v$, and increase in length and in tangential dimension as they move toward the site of union, $u$, with the shaft. From $u$ the ridges are carried axially from the base of the germ by growth of the shaft.

The axial growth of barbs and the shaft is composed of two phases: growth by cell division and growth by increase in the size of cells thus formed. Lillie and Juhn assume that a constant proportionality obtains between growth by cell division and growth by increase in the axial dimension of cells. We follow them in this assumption, and in text Figure 2 we shall suppose that we are dealing only with growth by cell division.

The region of growth by cell division is bounded in text Figure 2 by the heavy base line, $C_vC_d$, and the lighter line, $h_0$, through barb and shaft bases. We base this formal construction on the observation of Lillie and Juhn (1932), and of Greite (1934) that cell division occurs in part at least within the ridge proper. That portion of the zone of cell division lying between the ventral limit of the germ and the point of barb union with the shaft, $u$, includes only cells entering into barb primordia; that portion lying between $u$ and the dorsal mid-line of the germ gives rise to the primordium of the shaft (only half the shaft is shown in text Fig. 2). In terms of this representation the primordium of the shaft differs from the primordia of the barbs only in that it occupies a fixed position with reference to the zone of cell division and is of greater extent.

The multiplication of cells within the zone of cell division may lead (at least theoretically) to displacement of cells in three dimensions: axially, or at right angles to the base line, $C_vC_d$; tangentially, or in either direction parallel with the base line, $C_vC_d$; and radially, or at right angles to the plane of the zone as it is represented in text Figure 2.

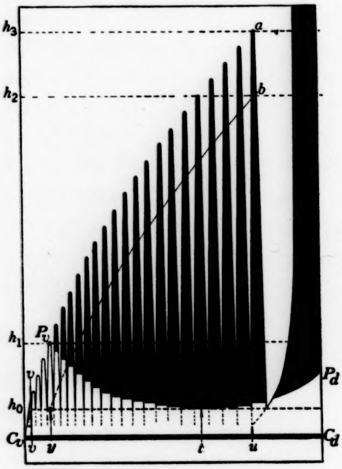

FIG. 2.—Diagrammatic representation of one collar limb of a regenerating feather germ. The germ has been split along the ventral superficial axis (left hand of figure) and also in the center of the shaft (right hand of figure); compare text Figure 8, *V*, ventral, and *D*, dorsal. Barbs and shaft are drawn at right angles to the base of the germ; this representation is strictly diagrammatic and should be compared with text Figure 14 and the germ reproduced as Figure 13, Plate V. The locus of primary growth by cell division is assumed to lie along $C_vC_d$; it extends axially to some level, $h_0$. The level at which barb bases appear is figured as a parallel with the base of growth by cell division; this is strictly diagrammatic. $P_vP_d$ represents a locus of simultaneous pigmentation tangent to $h_0$ at $t$. Ridges join the shaft at $u$. The c-isochrone parallel passing through the ventralmost barb on the p-isochrone, $P_vP_d$, is $h_1$. $P_vy$ indicates the level at which pigmentation occurs in the ventral region of the germ; ridges ventral to this ridge remain unpigmented until they reach $P_v$. The ridge-forming region is to the left of $v$–$v$. We assume that axial growth by cell division proceeds uniformly from $C_vC_d$, or between this base line and $h_0$. A point lying at $y$ follows the locus $yb$ and therefore remains equally distant from the apex $a$ of the completed barb. Ridge bases arising at the ventral surface, $v$–$v$, are transposed toward the shaft by the increasing diameter of every ridge in the collar and by the formation of additional ridges at $v$–$v$. Ridges uniting with the shaft primordium at $u$ are carried from the base of the collar; their spacing on the shaft is not shown.

The configuration of the c-isochrone requires that the axial component of growth be simultaneously uniform through the entire extent of the collar, including the shaft primordium. Any locus of reaction, $h_0$, parallel to $C_vC_d$, will thus be displaced uniformly from $C_vC_d$ on all barbs and the shaft during a given interval of time. Completion of growth of any individual barb and corresponding shaft level occurs simultaneously at some point, schematically indicated by $u$. A "mark" simultaneously imposed upon any barb and the shaft is therefore at completion of growth equally distant from the point of union, $u$, of that barb and the shaft. The relation of barb and shaft co-ordinates, $b = s$, found in the regenerated feather is thus accounted for by the assumption of simultaneously uniform growth by cell division in the axial sense.

The tangential (ventrodorsal) motion of ridges is referable to two observable evidences of growth: (*a*) ridges are formed continuously at the face of the ventral triangle, $v - v$; and (*b*) with increasing age of ridges the initial tangential dimension is increased. The cumulative growth represented by formation of ridges and the increase in tangential dimension of ridges determines the rate at which ridge bases reach the point of union, $u$, with the shaft primordium. In the fourth part of this paper we attempt to formulate the tangential motion of barbs more definitely in terms of growth vectors; for the present we need only account for the obvious fact that barbs, as individual elements of the germ, describe a ventrodorsal "motion" from site of origin to site of union with the shaft.

In connection with text Figure 1, it was pointed out that the locus of simultaneous pigmentation in the vane of the regenerated feather (represented diagrammatically by $P_vC_d$) is not, as a rule, identical with the locus of the c-isochrone, $C_vC_d$. The usual deflection of the pigmentation line is such that $b$ is greater than $s$, particularly near and at the margin of the line.

The relation of pigmentation lines and c-isochrones in the definitive feather corresponds to differing loci of reaction in the germ. With the locus of growth by cell division, $C_vC_d$, text Figure 2, as base, the locus of simultaneous pigmentation is represented (generally) by a curve, $P_vP_d$. This representation is strictly in accord with the observations of Lillie and Juhn; the differential displacement of the line of simultaneous pigmentation with reference to the assumed locus of growth by cell division is, however, apparently the result of other differentials than those immediately referable to the axial component of cell division.

Lillie and Juhn (1932) have shown that there is in individual barbs of the feather germ a gradient in reaction to certain physiological agents, such as thyroxin and the female hormone. This gradient is manifest in the extension of pigmentation or barbulation reaction from ridges near the shaft to ridges of the ventral triangle with increasing concentrations of the agents employed. Lillie and Juhn assume these differentials in reaction to depend for their realization and localization upon differing rates of processes arranged gradient-wise in the germ. They attempted to obtain a measure of these differentials by estimates of relative rates of axial growth of individual barbs at successive periods of development. One result of the present study is to show that rates of axial growth cannot be used as measure of the observed reaction differentials.

The reaction differentials with which Lillie and Juhn were concerned are based, as they are careful to point out, upon objective evidence which is not dependent upon differentials in the axial rate of barb growth on the one hand or upon any particular theory of development on the other. The relation of c-isochrones and lines of simultaneous pigmentation in regenerated feathers furnishes additional and exact evidence for the conclusion that pigmentation represents a ventrodorsal gradient of reaction with respect to the c-isochrone as base. We are not concerned in this paper with the specific reaction which may determine the gradient, but only with the geometric order of differentials between c- and p-isochrones.

## II. UNIFORM AXIAL GROWTH INCREMENTS

The evidence for uniform axial growth increments described in the Introduction comes mainly from the analysis of configurations in the regenerated feather.[4] The configurations with which we are primarily concerned here are the transversely extended lines of fault (Pl. I), the configuration of marginal contours at the apex (Pl. II), and contour configurations in the main vane of the feather (Pl. III).

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[4] We wish to express our cordial appreciation to Mr. Arthur Longini for his continued valuable criticism and generous participation in the analyses included in this as well as the other papers of this series.

The contour configurations are relations between spacing of barbs on the shaft and length of barbs.

The conclusions based upon a study of these configurations are strongly supported by analysis of pigmentation configurations. The main importance, however, of pigmentation configurations is in their bearing on organization of the germ; we have accordingly treated them in some detail.

We have attempted to relate the configurations in the regenerated feather as directly as possible to organization and development in the germ. The statement of growth relations given in the Introduction is the basis for interpretation of most of the results with which we are here concerned.

Methods for the preparation of material and for making measurements are described in the first paper of this series (Juhn and Fraps, 1936). All feathers are mounted with barbs at right angles to the straightened shaft, and all graphic representations will be referred to similar co-ordinates.

*Isochrones.*—Configurations which have been imposed simultaneously in all reactive elements of the regenerating germ will be denoted *isochrone* configurations or *isochrones*. This convenient term is due to Hardesty (1933), who applied it to describe in the germ or in the vane of the definitive feather "the locus of points which were formed at the same time." Hardesty used the term with reference to pigmentation lines only. The locus of simultaneous pigmentation in the germ, however, is but one of a number of loci of simultaneous reaction, determination, or "formation." We shall accordingly define the *isochrone*, whether in the germ or in the definitive feather, as *the locus of points of simultaneous and identical reaction or determination in homologous reactive centers of the germ.*

The c-isochrone has been defined in the Introduction as the locus of primary axial growth by cell division. We shall define *the locus of simultaneously imposed pigmentation* as the *p-isochrone. Loci of simultaneous fault reaction, or defect,* may be referred to as *f-isochrones.* We cannot be certain of the exact axial level at which fault reactions occur in the germ; the evidence presented later agrees, however, in locating isochrone fault reaction at approximately one or another parallel to the locus of growth by cell division. We must, nevertheless, make a clear distinction between the c-isochrone, which is defined as the locus of growth by cell division with respect to the axial dimension of growth, or an exact parallel with this locus, and the *approximation* to this configuration which characterizes the fault isochrone.

*Barb-shaft co-ordinates.*—It will be convenient to speak of the barb and shaft intercepts of any configuration, e.g., faults, and the point of union of any barb with the shaft as "barb-shaft co-ordinates." Transverse shaft levels are taken in apicobasal order as abscissas, and barb lengths in shaft-marginal order as ordinates. The term "barb-shaft co-ordinates" will then be the relation (generally the ratio) between a length or segment of barb and a corresponding length or segment of shaft entering into the definition of any configuration; see text Figures 3, 4, and 5.

*Uniform growth increments.*—We wish to make it very clear that we have used the term "uniform growth increment" (or "simultaneously uniform axial growth-rates") as a limiting condition which may or may not be realized in fact. The material at our disposal is not sufficiently precise to allow us to eliminate the possibility that small differentials in axial growth-rate (by cell division) do not exist around the base of the regenerating feather germ. Theoretically, it seems probable that such differences, of a small but well-defined order, may in fact differentiate one region of the germ from another.[5] The theoretical importance of differences in growth-rate, however small such differences may be, is evident from the investigations of Lillie and Juhn (1932). The main propositions of this paper follow as close approximations in any event.

### A. THE FAULT ISOCHRONES

Fault configurations are loci of defect in the structure of barbules, barbs, or shaft in the vane of the regenerated feather (Pl. I). Faults are of common occurrence in feathers and vary over a very wide range. Riddle (1908) and others cited by Riddle have described the typical fault structures, and we need note here only general characteristics.

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[5] We have in mind here growth differentials of the order implicit in D'Arcy Thompson's (1917) treatment of accretionary growth in terms of the logarithmic spiral (see also Huxley, 1932). Curvatures of shaft particularly may prove to be of considerable analytic value in this connection. The ventrodorsal reaction differentials of Lillie and Juhn most probably have a basis in growth differentials, and we are inclined to believe that such growth differentials will prove to fall within limits which are certainly not greater than would be required to account for shaft curvatures in terms of growth differentials.

The frequency of incidence of fault bars is approximately proportional to the rate of growth of the feathers bearing them (Riddle). They occur with greatest frequency in main tail feathers and in flights. In our material (Brown Leghorn mainly) faults of the type shown in Figure 2 of Plate I are more or less characteristic of the secondary flights. The fault is a narrow transverse band of barbule and barb "incision" or kinking; barbules are seldom missing. Barbs and shaft generally show the defect as a sharp depression, more evident, as a rule, on the outer surface of the feather than on the inner.

Fault bars of a rather different type are commonly characteristic of the tail feathers (Fig. 1, Pl. I). These defects often involve a considerable length of barb. Barbules are completely absent along parts of the theoretical transverse line of fault; barbules along other parts of the same line of fault are apparently normal.

Faults may thus be limited to the single collar limb or they may be localized within a collar limb. These localizations are undoubtedly of significance in terms of rates within the growing germ, but we shall not attempt an analysis of relations of this order.

Fault bars may be experimentally imposed upon the regenerating germ. Riddle induced typical fault structures by reduced feeding, feeding of Sudan III, mechanical "crumpling" with probable damage at the base of the germ, and administration of amyl nitrite. Kuhn (1932) produced faults by feeding deficiency diets and suggested also that the locus of reaction was in the zone of growth. We have induced bars in regenerating breast feathers through the action of adrenalin injected subcutaneously. The induced defect (Fig. 4, Pl. I) is a convex bending or arcing of barbs and shaft dorsally from the plane of the feather vane. The "bar" extends over a considerable length of barb, but barbule defects usually associated with extensive faults do not appear in these bars. The locus of incidence of the reaction falls, within very close limits, along the line of c-isochrone parallels. Figure 4 of Plate I does not show the approximately identical barb-shaft co-ordinates, inasmuch as the feather was photographed at an angle in order to bring out the definite bending of the barbs.

It should be clearly understood that actual lines of fault may vary greatly in barb-shaft co-ordinates from the exact value of the co-ordinates which define a c-isochrone, or a c-isochrone parallel. Thus in Figure 2 of Plate I, fault 7 obviously does not have the same barb-shaft co-ordinates as do faults 6 and 8. The upper and lower boundaries of faults 4 and 5, Figure 1 of Plate I, are likewise of different configurations in terms of barb-shaft co-ordinates.

In general, faults characterized by narrowness of extent upon barbs (and shaft), identity of defect (i.e., barbule defects solely or barb defect of the same order on each barb), and extending completely from margin to shaft, give also the closest approximation to the uniform co-ordinates of the c-isochrone. These characteristics are most probably the result of relatively severe reactions effective for a brief interval of time, as has been suggested by Kuhn (1932).

### 1. THE CONFIGURATION OF FAULT ISOCHRONES

The two important characteristics of the collar isochrone are symmetry in the two vane-halves and identity of barb-shaft co-ordinates. This definition rests on the determination of characteristics of actual configurations in regenerated feathers, and naturally it is never realized perfectly.

The relations subject to analysis are best seen by reference to Figure 3, Plate I. This feather (a major covert over a secondary, Brown Leghorn) bore a number of sharply defined but extremely attenuated "lines" of fault which are not visible after the feather has been mounted with paraffin. The lines of fault may be located, however, by cutting barbs at intervals along the line of defect before the feather is mounted. After the feather is mounted and photographed, diagonals are drawn from shaft to margin of the vane-halves along the lines of severed barb margins.

The co-ordinates of the lines constructed in this manner are determined by measuring the barb and shaft intercepts of the diagonals in terms of the representation given in text Figure 1. These are treated as ratios of barb and shaft lengths. Let *b* represent the length of barb segment from its point of union with the shaft to the point at which the diagonal representing the line of fault crosses it, and $s$ the length of shaft segment from the base of the selected barb to the intersection of the line of fault (as drawn) with the shaft. If $b/s = 1$, the line of fault is, within the limitations of method, identical with the c-isochrone as we have defined it. Ordinarily, $b/s$ will not be exactly equal to unity. The deviations of faults of this type from the c-isochrone are usually small, and they are not consistently in one direction.

The lines drawn into Figure 3 of Plate I are constructed at the theoretically exact angle of the c-isochrone. It is evident at once that in this particular case the actual fault lines, excepting only the sharply broken fault 5 (cf. text Fig. 4), do not vary appreciably from the exactly defined c-isochrone.

A more accurate procedure is to determine by measurement the co-ordinates of lines of fault which are visible after the feather has been mounted. We measure, first, the distance of each barb in basal direction from some point slightly apical to the point of incidence of the fault on the shaft, and secondly, the distance from the point of incidence of the fault on each barb to its point of union with the shaft.

The barb-shaft ratios are calculated for every barb carrying the fault, or the data are treated graphically. The data considered here are best presented in the form of graphs.

Text Figure 3 represents the barb-shaft co-ordinates of an "arc" bar (Pl. I, Fig. 4) in a secondary major covert. The barb co-ordinates are located from the base of the barb to the center of the arc forming the fault; the centers of the bent portions of each barb are remarkably well defined and can be located with a high degree of accuracy.

The points of fault incidence on successive barbs in text Figure 3 are represented by disks on the ordinates. The c-isochrone parallel, *VD*, is constructed through the points of fault incidence; points of incidence of *VD* on the shaft and on any barb are therefore equidistant from the point of union of each barb with the shaft. It is clear that the actual points of fault incidence vary but little from the theoretical c-isochrone parallel. One might possibly show by mathematical treatment that for the data given in text Figure 3 there is a small deflection of the points of incidence of the fault toward the margin of the vane-half, but other determinations, comparable in every respect, show slight deflections in opposite sense.

The exact relation with which we are dealing should be made clear in terms of growth of the germ. Regardless of the level at which fault incidence occurs in the germ, the fault represented in text Figure 3 is well defined on the shaft and falls very closely along the c-isochrone parallel, *VD*. Let $a_2u_2$ represent any barb crossed by the line of fault incidence, and let $w_2$ represent the point of fault incidence on this barb. The experimentally determined relation is that $w_2u_2 = u_2D$. Similarly, $w_1u_1 = u_1D$. With reference to the initial line of fault as base, every barb carrying the fault increases equally in length with the shaft until it has joined the shaft. In terms of text Figure 2, a fault which we may assume to have been imposed at the level $h_0$ (or indifferently at $h_1$) must be removed from the base of the germ by exactly the amount as is the corresponding fault on the shaft before union of barb and shaft occurs.

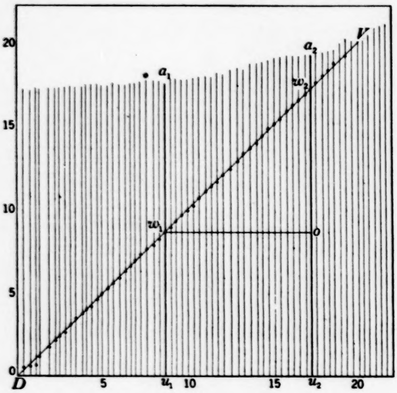

FIG. 3.—Points of incidence of a fault bar on barbs and shaft in the right vane-half of a covert of a secondary, Brown Leghorn capon. Abscissas, distances in basal direction in millimeters from the point of incidence of the fault on the shaft ($D = 0$); ordinates, lengths of barbs in millimeters from points of union with the shaft. The diagonal, *VD*, is constructed at the theoretically limiting angle with the line of union of barbs and shaft; for this condition $Du_1 = u_1w_1$, $Du_2 = u_2w_2$, etc.

The fault represented in text Figure 3 is clearly defined on the shaft, as we have observed earlier. If, however, faults were never visible on the shaft, we should be able to demonstrate that equal increments are added to barb and shaft from the base line of growth in the same time interval by analysis of the relation between distances separating barbs and the locus of fault incidence on barbs. In text Figure 3,

$u_1u_2$ is of course equal to $w_1o$, and $w_2u_2 - w_1u_1 = w_2o$, from which it follows that $w_1o = w_2o$. This is to say that the *difference* in lengths of two barbs from the point of fault incidence to the point of union of those barbs with the shaft is equal to the distance separating the same barbs at the shaft. We can understand this relation to mean only that equal increments are added to barb $a_2u_2$ *and the shaft* after barb $a_1u_1$ has joined the shaft at $u_1$.

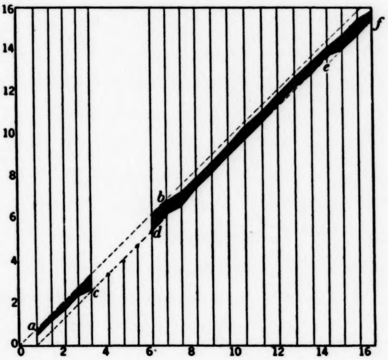

FIG. 4.—Discontinuous fault in the vane of a hackle feather, Brown Leghorn capon. Abscissas, distances on shaft in millimeters; ordinates, distances on barbs from shaft in millimeters. The segment *ab* represents the initial level of fault reaction, segment *cd* an intermediate level, and segment *ef* the basalmost level. The marginal limit of barbs lost between *c* and *d* fall very exactly along the c-isochrone.

The approximate symmetry of fault configurations in opposite vane-halves is evident in such feathers as that shown in Figure 4, Plate I. In many instances faults involve the shaft with sufficient precision to make it clear that there is no appreciable displacement of the point of fault incidence on one side of the shaft as compared with the other. The same conclusion is borne out if we calculate the points of shaft intersection of lines of best fit drawn through points of fault incidence on barbs according to the procedure described in connection with text Figure 3. Lines so constructed for faults in opposite vane-halves are found to intersect the shaft at levels differing by very small amounts on the two sides, and in our material we have been unable to find any consistent difference in transverse level of incidence. The limitations of material and method applying to the definition of co-ordinates in each vane-half separately naturally hold also in location of the level of shaft intersection. We are unable to exclude, therefore, the possibility of a slight but real difference in the level of fault isochrone intersection with the shaft.

Fault bars in many instances are "discontinuous" with respect to a single c-isochrone locus. Fault 5 of Figure 3, Plate I, is an example of this discontinuity. Text Figure 4 represents a similarly discontinuous fault in one vane-half of a hackle feather, Brown Leghorn capon. Abscissas and ordinates give distances in millimeters from the point of intersection with the shaft of the c-isochrone drawn approximately along the initial region of fault, *ab*.

The several "levels" of fault fall approximately along c-isochrones, as is evident by comparing the boundaries of the heavy black areas, representing total extent of barb defect, with the dotted lines. The most intense region of reaction appears to have been from *c* to *d*. The marginal portions of three barbs in this region are missing; the lengths of the central segments are indicated by the three disks falling along *cd*. A sharply defined incision appears at *c* and another at *d;* all effects between *c* and *d* undoubtedly represent the locus of maximum intensity of the fault reaction.

Fault configurations of this type are clearly evidence for reaction differentials in the collar. The region of fault from *a* to *b* must be due to reaction either at an earlier time or at a higher level on the ridges; similarly, the reaction effecting the fault continued for a longer time along *ef*, or cells closer to the base line of growth were affected through this limited region. The fault represented in text Figure 3 is, in contrast with this situation, practically identical on every barb. This we take to be evidence for relatively intense and simultaneous reaction; it is not evidence that no reaction differentials exist in the collar, a point which we wish to make expressly clear.

### 2. ABSOLUTE AND RELATIVE BARB-SHAFT CO-ORDINATES

The subcutaneous injection of thyroxin alters the normal spacing of barbs on the shaft in many types of feathers (Juhn and Fraps, 1934*b,* Hardesty, 1935). The *absolute* values of the barb-shaft co-ordinates of c-isochrones through modified regions must therefore be altered also (see p. 359). The data presented graphically in text Figure 5 show that the locus of fault through the vane-halves remains unaltered. Points of fault incidence on barbs are represented as ordinates; distances on the shaft from point of incidence of the fault with the shaft, as abscissas. The fault is well defined on the shaft at *D* (=0 on abscissa). The fault does not extend completely to the margin of the feather but is well defined on barbs 25 mm. in length. The c-isochrone parallel through the points of fault incidence is represented as $VD$.

Barb frequency is defined as the reciprocal of the distance (in mm.) between two barbs and is plotted against shaft lengths in text Figure 5 as the broken curve, $f_s$. It is clear that the relation, $b = s$, characteristic of the c-isochrone, or c-isochrone parallels, is maintained independently of the distribution of barbs on the shaft; $uw = uD$, or approximately so, for every barb bearing the fault.

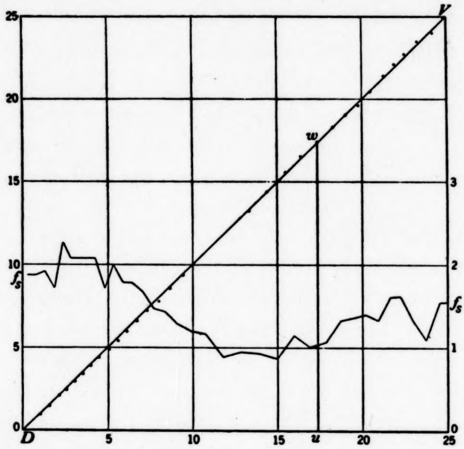

FIG. 5.—Line of fault through the vane of a hackle feather in which spacing of barbs has been experimentally modified (thyroxin). Abscissas, distances on shaft in millimeters from shaft intercept of the line of fault; ordinates, left, distances on barbs from shaft in millimeters; ordinates, right, number of barbs per millimeter on shaft (curve $f_s$). The black disks indicate points of incidence of the fault on each barb; the line of fault is not altered by the spacing of barbs. The relation $Du = uw$ remains constant for all barbs and corresponding shaft segments.

In a normal hackle feather the spacing of barbs through the region represented in text Figure 5 would be fairly uniform, and $f_s$ would range around 1.5 barbs per millimeter. Since from any point of origin, as $D$, we have a number of barbs more closely spaced than is normal from 0 to 5 mm. (abscissa), it follows that these barbs must have grown by less than their normal length before joining the shaft.

In the region from 10 to 15 mm. on the abscissa the barb frequency is considerably less than normal; and corresponding with this, barbs have grown to a greater length than would otherwise have been the case. It is unnecessary to calculate the exact magnitudes involved here; the important point is that the ratio of barb and shaft co-ordinates, $b/s = 1$, is maintained within rather close limits independently of the spacing of barbs on the shaft and independently, therefore, of the *absolute* length of barb and shaft segments.[6]

### 3. THE LOCUS OF FAULT REACTION

The only direct evidence on the locus of fault origin in the germ is due to Riddle, who states that the first indication of fault formation is "a loose union of scattered cells in that part of the *intermediate* cell layer which is forming the barbule cells" (1908, p. 335). The significance of this statement is evident, since it refers the locus of fault reaction directly to the limited region of growth by cell division at the base of the feather germ.

The interference with the growth processes which is commonly recorded as fault bars in the vane of the definitive feather is due, according to evidence presented by Riddle, to reduction of the nutritional level in the regenerating follicle. Riddle associated the reduced level with lowered blood pressure during the night hours of inactivity. Fault bars not infrequently recur with considerable regularity in the feather vanes, the distances separating successive bars corresponding approximately with the expected daily growth of the feather.

It is probable that fault-bar formations generally represent special instances of the principle of thresholds formulated by Lillie and Juhn (1932). Juhn and Gustavson demonstrated (1930) that the quantity of female hormone required to effect a given reaction in different plumage areas is a function of the axial growth-rates of the feathers within those areas. Relatively high concentrations of the hormone were required to induce female pigmentation patterns in rapidly growing feathers; lower concentrations induce the same reaction—without causing reaction in rapidly growing feathers—in feathers of lower growth-rates. Reversion to normal pigmentation occurs after shorter duration of the induced reaction in rapidly growing feathers than in slowly growing feathers. The order of reversion with respect to hormone concentration thus varies directly with the axial rate of growth of the regenerating feather.

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[6] In the material at our disposal (Brown Leghorn capon) we have not a single instance of well-defined faults in feathers which have been subject to relatively high concentrations of thyroxin. It is certainly not improbable that sufficiently high concentrations of thyroxin (or other agents) would bring about alteration in the barb-shaft co-ordinates of faults previously imposed, if only through effects differentially recorded in the second phase of growth.

The frequency of incidence of faults is at least roughly proportional to the axial growth-rate of regenerating feathers, as noted above. Fault bars represent certainly a "reversion" from the normal processes of growth and differentiation of one or another structural element. The order of reversions of this type suggests that fault reactions are referable directly to the principle discovered by Juhn and Gustavson and formulated by Lillie and Juhn, and thus in all probability they involve cell division directly.

Evidence supporting this conclusion comes also, if indirectly, from the experiments of Kuhn (1932) on regeneration of feathers during feeding of deficiency diets. Kuhn fed polished rice which induced defects or "shocks," and brought about recovery by feeding yeast. If the polished-rice diet is continued over a sufficiently long period, there is a definite sequence of interruptions in the normal processes of growth and determination. The formation of the "radioli" is first interrupted, later the formation of barbules with barb defects in the same position, and finally with increasing "shock" no barb cells are differentiated through the affected cross-section. Normal feather regeneration is brought about by feeding yeast.

It is not necessary to suppose that all apparent "faults" represent reversion from a normal level of growth (or determination). It is at least theoretically possible that configurations with typical c-isochrone co-ordinates might be imposed by a sudden acceleration of the processes of growth. The faults induced by adrenalin injection (Fig. 4, Pl. I) are easily imposed upon breast feathers, which ordinarily show very few faults. If the induced reaction accelerated some phase of development sufficiently, we should expect faults to appear first in feathers characterized by slow growth-rates. In either case the origin of altered structures, defective or otherwise, as a result of altered growth-rate in the embryonic cells of the collar appears to be a special case of the general principle described by Juhn and Gustavson, and as such can be referred most probably to the region of growth by cell division at the base of the germ.[7]

Hardesty (1935) has recently reported the presence of fault bars (barbule defects) which extend across guinea feathers along the usual narrow transverse band. The barbules along such bars are poorly developed, or they may be absent altogether; and in some cases the defect involves the barb also. She notes that the faults are more common and of greater extent following thyroxin treatment, and also that the degree of defect increases with increasing concentration of thyroxin. Hardesty attempted to locate the region of fault in the feather germ with reference to the locus of pigmentation. According to her, "the barbule effect occurs slightly above the line of pigmentation and approximately along an isochrone line" ("isochrone line" refers here to our p-isochrone). She observes that the line of barbule defect is continuous (i.e., symmetrical at the shaft) in asymmetrical feathers and is therefore not symmetrical with respect to corresponding p-isochrones of the regenerated feather.

Hardesty does not present in detail the observations upon which this conclusion is based. If, in fact, the line of barbule defect is above the line of pigmentation, and if, moreover, the defect is due to interference with normal cell division, it seems probable that cell divisions must extend into the region of pigmentation. This conclusion would not be in accord with the statements of Lillie and Juhn (1932) or of Greite (1934), that pigmentation occurs at or near the level at which cell division ceases. The alternative conclusion would be that the fault bar, at least in this particular instance, represents failure of the processes of differentiation rather than of cell growth, or possibly some actual destruction of formed elements, i.e., barbules.

The locus of fault reaction in the germ may theoretically lie through the basal region of embryonic cell division, or reactions determined in this level may become apparent only at higher levels. In certain instances the locus of fault reaction may be specifically the level of barbule formation (Riddle); in others it may lie at considerably higher levels, cutting portions at least of pigmentation isochrones (Hardesty).

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[7] Similar considerations probably hold also for the "fundamental bars" of Whitman (see Riddle, 1908), if these are in fact evidences of a diurnal rhythm in axial growth-rate of the regenerating feather.

In any event, the limiting configuration of faults generally is defined by equal barb and shaft co-ordinates; and regardless of the absolute level of incidence in the germ, the loci of reaction are represented in the vane of regenerated feathers as a system of parallels. The entire system of parallels can most readily be understood by referring them to annular parallels within the zone of simultaneously uniform axial growth by cell division, as $C_vC_d$, $h_0$, $h_1$, text Figure 2. As limiting configurations, the c-isochrone system of parallels is thus evidence for simultaneously uniform axial growth increments by cell division.

### B. LIMITING VALUE OF MARGINAL CONTOURS

The marginal contour of the regenerated feather is the continuous line joining the apexes of barbs from apex to base of the feather. In the present discussion we suppose that barbs are mounted at the usual angle of 90° with the shaft, and the c-isochrone has therefore the angular construction of 45° with the line of union of barbs and shaft.

Contour configurations commonly found at the apex of normally regenerated feathers and at subapical levels of feathers regenerated under experimentally modified hormone conditions furnish independent evidence for simultaneously uniform growth increments. In the limiting instances, contour configurations of barb apexes are referable to barb-shaft co-ordinates in the regenerated feather which are identical with the co-ordinates of the c-isochrone as this has been defined previously. It should be emphasized that we are dealing here with limiting configurations.

*Contour tangents* are defined as tangents drawn to the marginal contours of regenerated feathers mounted as described. Such tangents may, of course, be drawn with reference to any point on the curve of marginal contour of a feather, as *ab*, Figure 3 of Plate I, tangent to the marginal contour at the point *p*. We are interested mainly in contour tangents which form an angle approximating 45° with the line of barb-shaft union, the vertex of the angle being directed apically (Pls. II and III).

### I. LIMITING VALUE OF CONTOUR TANGENTS AT THE APEX OF THE FEATHER

Apical portions of three feathers are reproduced in Plate II, Figures 5, 6, and 7. Figures 5 and 7 represent equal enlargements of the originals; Figure 6 represents one-half the enlargement of Figures 5 and 7.

*Initial* or *apical* c-isochrones are c-isochrones drawn from the apexes of the primary barbs when these lie in the line of barb-shaft union. These are indicated by "0" in the figures of Plate I; c-isochrones "10" in the same figures are drawn from shaft intercepts 10 mm. from apexes of the primary barbs.

The limiting configuration at the apex is that in which a number of barb apexes fall along the line of initial c-isochrones. These are the apexes between apexes of the primary barbs and points indicated approximately by *i* in Figures 5 and 7 of Plate II. Many feathers are characterized by "acute" apexes in contrast with the right-angle formations shown in Plate II, Figures 5 and 7; typical of these is the hackle feather (Brown Leghorn capon) reproduced as Figure 6, Plate II. Very few barb apexes lie along initial c-isochrones in extremely "acute" formations.

Of more significance in the present connection is the fact that (in our experience) the 45° contour tangent—the initial c-isochrone—represents the limiting configuration, in that barb apexes below the primary barbs may lie along this isochrone but never extend through it. We shall refer to "limiting" apical configurations strictly in this sense.

The significance of the limiting apical configuration is made clear by an important observation due to Lillie and Juhn. These authors pointed out that two distinct phases are to be recognized in formation of the initial complement of ridges in the collar. During the first phase a number of ridges are formed approximately simultaneously; Lillie and Juhn estimate that in the case of the Brown Leghorn breast feather "about 25 barbs form practically simultaneously on each side and that the remainder arise *seriatim* in the ventral field" (1932, p. 139). During the second phase of ridge origin, individual ridges arise serially from the lateral surfaces of the ventral triangle (text Fig. 2.)

Actually, we cannot know that ridges of the first phase are laid down simultaneously, since theoretically any number of ridges may be formed in a definite time order in the wall of the germ before initiation of axial growth. It is nevertheless probable that a certain number of apicalmost ridges originate simultaneously, in agreement with the observation of Lillie and Juhn that germs in the earliest stages of ridge formation may show either no ridges or a considerable number of ridges. These are the ridges which, as barbs in the regenerated feather, define the limiting contour at their apexes.

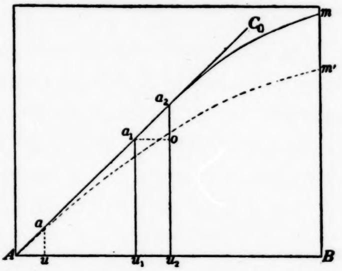

FIG. 6.—The geometric relations involved in the limiting contour formation at the apexes of feathers. $AB$, line of barb-shaft union; apex of the primary barbs at $A$. Barbs are represented by $a_1u_1$ and $a_2u_2$; assumed length of the primary barb, $au$. $C_0$ is the initial c-isochrone (c-isochrone o of plates). Limiting marginal contour, $Am$; "acute" marginal contour, $Am'$. The limiting value of barb-shaft co-ordinates is that $Au_1$ shall equal $a_1u_1$.

The relations involved in the limiting configurations are (growth) relations between barb and shaft lengths and are given in text Figure 6. The apexes of the primary ridges are represented at $A$; the basal level of the vane-half section at $B$. Marginal contours are represented by $Am$ and $Am'$. Barbs $a_1u_1$ and $a_2u_2$ are drawn at right angles to the line of barb-shaft union, $AB$; the initial c-isochrone, or limiting contour tangent, is represented by $C_0$.

In the limiting configuration, the barb apex, $a_1$, falls along the contour tangent, $C_0$. Let $u_1$ represent the point of union with the shaft of the barb $a_1u_1$. It follows that $Au_1 = a_1u_1$. If we assume that barb apex $a_1$ was laid down simultaneously with apexes of the primary barbs at $A$, it follows that the *total* growth (and presumably also rate of growth by cell division), of the barb $a_1u_1$ is exactly equal to the total axial growth of the primary barbs, $Au$, plus the definitive shaft segment $uu_1$.

The limiting barb-shaft relation at the apex of the feather can be generalized as follows: *The length of any barb in the simultaneously formed initial complement is equal to the length of the primary barbs plus the length of shaft segment lying between the base of the primary* *barbs and the point of union of the given barb with the shaft.* This relation is completely accounted for on the assumption of simultaneous origin of barb apexes falling along limiting contour tangents, and uniform axial growth increments at points of correspondence in barb and shaft. We have pointed out earlier that we cannot be absolutely certain of simultaneous origin of barb apexes, but we come to exactly the same conclusion if we assume the apexes of barbs falling along the initial c-isochrone, $C_0$, to have been laid down before initiation of axial growth.

Lillie and Juhn consider the shaft proper to begin at the bases of the two primary ridges, and we have recognized this distinction in the foregoing relation of barb-shaft co-ordinates. The distinction is clearly true of the shaft as a primary structure. Greite (1934), however, states that the site of the future shaft is definitely localized before its formation as an independent structure, and he notes the reversed symmetry of barbulation on the two primary ridges as evidence for this conclusion. In calculating the barb-shaft co-ordinates of simultaneously formed apical barbs, we consider the two primary barbs to be components of shaft length. This procedure does not necessarily imply that the shaft represents simply fused barb bases (cf. Strong, 1902); it merely locates the point with reference to which the length of "shaft" is identical with the length of simultaneously formed barbs subsequently joining the shaft.

The validity of our interpretation of limiting apical configurations is not, however, dependent upon the relation involving apicalmost barbs only, since we can arrive at the same geometric (and growth) relations in terms of any two barbs the apexes of which lie along the initial c-isochrone. Let $a_1o$, text Figure 6, be the distance between two barbs at their points of union with the shaft, $u_1$ and $u_2$. The difference in lengths of these barbs is $a_2o$. But since the initial c-isochrone, $C_0$, is drawn at 45° to $AB$, it follows that $a_1o = a_2o$; that is, the difference in length of barbs $a_1u_1$ and $a_2u_2$ is equal to the length of shaft, $a_1o$ (or $u_1u_2$), between the points of union of these barbs with the shaft. This relation is directly accounted for if points of correspondence in the two barbs and the shaft, i.e., on all c-isochrone parallels, increase in length by simultaneously uniform increments.

If barb apexes fall along an "acute" marginal contour, *Am′*, text Figure 6, the difference in length of any two barbs is less than the length of shaft between the points of union of these barbs with the shaft. We take this difference to mean that barb apexes composing marginal contours other than limiting contours have been formed in a definite time sequence, only the two primary barb apexes having been laid down simultaneously.

Marginal contours resulting from the serial and individual formation of barb apexes in dorsoventral order are characterized by contour tangents of greatest slope nearest the primary barb apexes. The *rate* of barb formation, then, proceeds most rapidly in the region of the site of the primary ridges, decreasing with completion of the collar complement (text Fig. 9, curve 3, p. 348). The acute apical contour is therefore due to a quantitative variant in the same relations which define the limiting contour tangent.

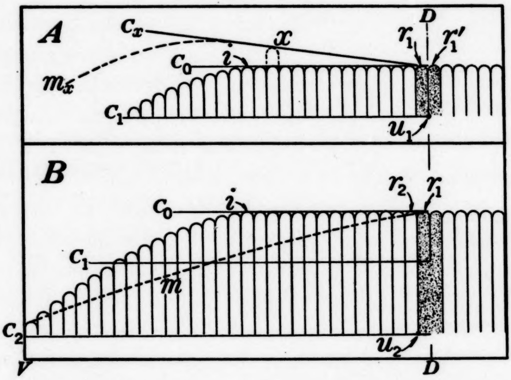

FIG. 7.—Diagrammatic representation of the germinal relations involved in formation of limiting apical configurations; *A* represents a somewhat earlier period than *B*. Ridge apexes formed simultaneously lie along $C_0$; $r_1$ and $r_1'$, primary ridges; *i*, the ventralmost ridge of the initial collar complement. The union of primary ridges to form the shaft proper occurs at $u_1$ along the collar isochrone $C_1$. Similarly, the next barb joins the shaft at $u_2$ along the c-isochrone $C_2$. Compare with text Figure 8.

The germinal relations which correspond to the apical contours of regenerated feathers are represented diagrammatically in text Figures 7 and 8. In text Figure 7*A* we suppose ridges to have formed simultaneously in opposite directions from a primary pair of ridges, $r_1$ and $r'_1$. These are the ridges lying between *i* and $r_1$ in the left collar limb, and the apexes of which fall along the initial c-isochrone, $C_0$. If we assume that some small degree of axial growth has been accomplished at this stage of development, we may represent the locus of growth by cell division by c-isochrone $C_1$. Ridges to the left of *i* are figured as of decreasing length in correspondence with increasing time intervals separating the origin of their apexes.

Excepting only those ridges formed simultaneously, ridges originate in dorsoventral order until the collar has a complete complement; the extension of ridges around the wall of the germ is therefore from the dorsal axis, *D–D*, toward the left-hand limit of the diagram. The *motion* of individual ridge bases is the reverse of the order of ridge formation, i.e., from ventral to dorsal collar positions.

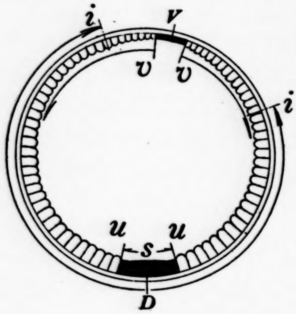

FIG. 8.—Transverse section at the base of the feather germ, diagrammatic. *D*, dorsal limit of the germ; *V*, ventral limit. In formation of the initial complement, barbs are laid down from *D* to *i* in each collar limb. In the main vane region barbs are laid down at *v;* tangential growth carries barbs from *v* to *u* in each collar limb. The shaft primordium is indicated by *S*. This region is initially defined as the point of union of the primary ridges; it increases in the direction of the arrows at *S*, and at the base of the feather comes to involve the entire circumference of the collar.

The relations shown in text Figure 7*A* are emphasized by reference to text Figure 8, which represents a transverse cross-section at the base (approximately the level $h_0$ of text Fig. 2) of a regenerating feather germ after formation of complete collar complements. The ventral superficial axis is at *V;* the dorsal superficial axis, at *D*. At the stage of development assumed for the figure, the ventral triangle (between the ridges, *v* and *v*) and the shaft primordium, *S*, are definitely localized.

The initial collar-limb complements are laid down in the wall of the germ from *D* to *i*, either simultaneously or in a definite time order. If the ridges are formed simultaneously, *i* of text Figures 7 and 8 will correspond with *i* on the limiting marginal contours of

Plate II. The completion of the collar complement (from *i* to *v*, text Fig. 8) proceeds at a rapid, but decreasing, rate (cf. text Figs. 9 and 10). After completion of the collar complement, ridges form serially from the lateral surfaces, *v*, of the ventral triangle; and the individual ridge bases "move" from *v* to *u*, at which point they join the shaft and are carried out of the collar.

Given an initial collar complement of simultaneous formation (text Fig. 7*A*), we must account for the limiting apical configuration in the regenerated feather. We must also recognize that the limiting configuration of apical contours is independent of distances between barbs on the shaft, a point sufficiently emphasized by differences in spacing of barbs on the shafts of the feathers reproduced as Figures 5 and 7, Plate II (these feathers are equally enlarged).

If the apexes of simultaneously formed ridges (those between *i* and $r_1$) have been laid down along a line parallel with the lines of simultaneous growth, $C_1$ and $C_2$, text Figure 7, and axial growth (by cell division) is simultaneously uniform through all stages of growth, no displacement of the initial c-isochrone, $C_0$, can be effected. This isochrone will continue to rise as a parallel above the zone of growth by cell division.[8] After a definite interval of axial growth, the two primary ridges, $r_1$ and $r'_1$, fuse at their bases, $u_1$, to compose the apex of the shaft proper (Fig. 7*A*). The point of union of the primary ridges is similarly raised above the locus of growth by cell division ($C_2$, Fig. 7*B*) to exactly the same degree that lengths are added to all ridges lying along the base isochrone $C_1$ of Figure 7*A*. When the second barb (in the left collar limb) joins the shaft at $u_2$ (Fig. 7*B*), the length of this barb will be exactly equal to the length of the primary ridges plus the length of definitive shaft lying between $C_1$ and $C_2$. If the simultaneously uniform growth relation is maintained, the apexes of all barbs of the simultaneously originating complement must thus come to lie on the limiting contour tangent.

If the representation shown in text Figure 7 corresponds with actualities in the regenerating germ, it is clearly immaterial at what level union of the primary ridges, $r_1$ and $r'_1$, occurs, or at what level any of the simultaneously originating complement of ridges shall join the shaft. We have pointed out earlier that the locus of fault reaction (simultaneously imposed on all growing elements) is likewise independent of the spacing of barbs on the shaft or of the *absolute* barb-shaft co-ordinates which define that locus in the regenerated feather.

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[8] It is necessary to realize that we are speaking here of growth increments due to cell division; the second phase of growth is apparently subject to differentials which temporarily "distort" the c-isochrone parallel, but at completion of this phase of growth the original relation of parallels is restored.

The simultaneous origin of ridges, whether at the apex of the feather or at other levels, is obviously the limiting "rate" of ridge formation. The initial collar complement need not, however, be formed simultaneously. Assuming that axial growth is under way at the time of formation of the primary ridges, and that ridge apexes form at the same level with respect to the locus of growth by cell division, the serial origin of ridges ventrally from the primary ridges is represented by the contour, $m$, text Figure 7*B*. The level at which $m$ cuts the ridges of the previously described simultaneously formed complement is the locus of the serially formed apexes. The distance between $C_0$ and $m$ along the axes of successive ridges in dorsoventral order represents exactly the length to which the primary ridges have grown at the time of formation of sucessive ridges. The uniform axial rate of growth of formed ridges by cell division and increasing time intervals between origin of successive ridges thus account for the acute marginal contour of the regenerated feather.

One additional point should be touched on briefly in connection with the simultaneous formation of ridges. If the collar is subject to differentials in axial growth-rate (by cell division), the apexes of ridges in regions of high growth-rate (as at $x$, text Fig. 7*A*) should come to lie outside the initial c-isochrone with completion of growth. For the relations represented in text Figure 7*A*, the apical contour tangent corresponding with $m_x$ should make an angle of more than 45° with the line of barb-shaft union. We have not yet found such a situation, although we cannot rule out the possibility that small (but not insignificant) differentials of this order do in fact exist (see p. 328). Also, some care must be exercised in the selection of feathers in order to avoid damaged apexes; it is likely that normal "abrasion," however, would tend to accentuate rather than mask contour tangents characterized by angles greater than the usual 45° c-isochrone.

Apical formations are of particular interest in view of the constancy of the limiting contour tangent in feathers of widely differing types, a point noted in connection with the great difference in spacing of barbs in the feathers reproduced as Figures 5 and 7, Plate II. The order of differences in magnitudes entering directly into apical relations shown by these feathers (and a hackle) are represented graphically in text Figures 9 and 10. The necessary data are obtained by application of c-isochrones at the apex and at 1-mm. intervals through 10 mm. from the apex. Methods and procedures are described in the first paper of this series (Juhn and Fraps, 1936).

The curves of text Figure 9 represent cumulative barb numbers at marginal intercepts (solid lines) and at shaft intercepts (broken lines) of successive c-isochrones. The barb numbers are totals for left and right vane-halves in all instances. Distances in millimeters from apexes of primary barbs are given as abscissas; corresponding barb numbers, as ordinates. Simultaneously determined complements are indicated by *i*.

Marginal barb numbers (solid lines) are a measure of the total number of barb apexes which actually form, after completion of all growth, at a given axial level of growth; shaft barb numbers are the total number of barb bases completed at a given level of axial growth.

Curves 1 (solid and broken) are obtained from the Barred Rock feather reproduced as Figure 5 of Plate II; curves 2 (solid and broken) are from the guinea fowl feather reproduced on the same plate. The third set of curves represent the same data for the apical 10 mm. of a hackle feather (not that shown in Pl. II).

Figures 5 and 7 of Plate II are equally enlarged and are therefore directly comparable. In spite of the much more "blunt" appearance of the guinea-fowl feather, the Barred Rock germ actually formed a much larger simultaneous complement (*i* of text Fig. 9); furthermore, the Barred Rock germ continued to form a larger number of barbs (apexes) per millimeter of axial growth than did the guinea germ. But the number of barbs completed at corresponding levels of axial growth is also much greater in the Barred Rock than in the guinea fowl (cf. curves 1 and 2, text Fig. 9). It is largely in consequence of the relatively great degree of axial growth accomplished during the interval separating the union of successive barbs with the shaft that the guinea feather shows the combination of widely spaced and relatively long barbs from a simultaneous complement initially smaller than that of the Barred Rock. We apparently have here a set of growth relations (axial and tangential) of first-class importance, and possibly also of interest genetically. For the moment, however, it is sufficient to note only the constancy of the limiting marginal contour in feathers differing so markedly in other respects.

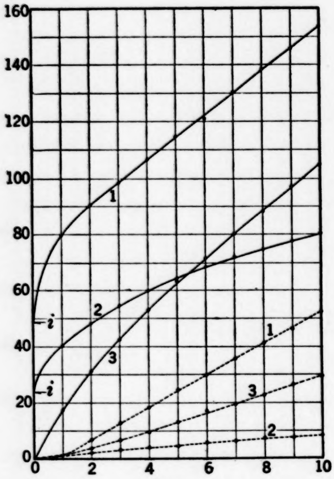

Fig. 9

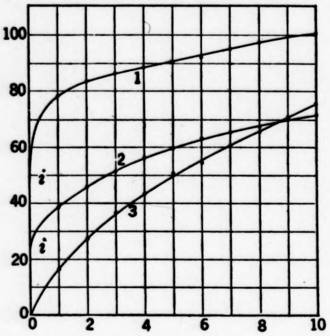

Fig. 10

Fig. 9.—Cumulative barb numbers, both vane-halves; for the marginal intercepts, solid lines; for the shaft intercepts, broken lines. Abscissas, distances in millimeters from apexes of the primary barbs; ordinates, barb numbers. Curves 1 (solid and broken), breast feather, Barred Rock ♀, Figure 5 of Plate 2. Curves 2, breast feather, guinea fowl ♂, Figure 7 of Plate II. Curves 3, hackle feather, Brown Leghorn capon (not that represented on Pl. II).

Fig. 10.—Number of barbs on successive and opposed c-isochrones. Abscissas, distances in millimeters from apexes of the primary barbs; ordinates, barb numbers. The curves are for the same feathers described in connection with text Figure 9.

The curves for the hackle feather require no particular description further than to call attention to the form of the curve for marginal barb numbers (3, solid line, text Fig. 9) in contrast with the corresponding curves for limiting configurations.

Curves for numbers of barbs lying on successive and opposed c-isochrones for the three feathers described in connection with text

Figure 9 are given in text Figure 10. The number of barbs thus defined may be taken as the number of barb "loci" simultaneously in process of growth by cell division (Juhn and Fraps, 1936). In these terms the Barred Rock germ (curve 1, text Fig. 10) comes most quickly to approximate "equilibrium"; the guinea germ (curve 2) does so somewhat more slowly. The hackle germ attains its complete barb complement very slowly, but it will be observed that the number of barbs in process of growth (as thus measured) becomes greater than the corresponding number in the guinea germ at a relatively early stage (*ca.* 9 mm. axial level).

### 2. LIMITING VALUE OF CONTOUR TANGENTS AT SUBAPICAL LEVELS

If the relations described for apical configurations are general, we should expect to find that the limiting marginal contour at any level of the shaft is defined also by tangents which are identical in slope with the slope of limiting apical tangents. This expectation could only be realized if barbs might be formed simultaneously after development of the feather is well under way.

That the theoretically possible limiting configuration is in fact realized, or approximately realized, is evident in experimentally induced marginal contours in saddle feathers. The feather reproduced as Figure 8, Plate III, is from the saddle tracts of a Brown Leghorn capon; the apical portion, from the apex to c-isochrone 20 (at 20 mm. from apex), was regenerated under normal physiological conditions. At or near the 20-mm. level the bird was injected with 10 mg. of thyroxin. A normally regenerated saddle feather is reproduced as Figure 9 of Plate IV, and serves as a control.

It is evident, by examination of the close approximation of barb apexes to c-isochrones 24 (left and right vane-halves, Plate III), that the limiting contour tangent previously described in connection with simultaneous apical formations has been very closely approximated in this feather under the action of thyroxin. It is to be observed also that barb apexes in the opposite vane-halves fall upon opposed c-isochrones, although the absolute lengths to which these barbs have grown is very different in opposite vane-halves, and shows, in fact, a reversal in the length relations obtaining before thyroxin action.

The geometric representation of the limiting marginal contour relation at subapical levels is shown in text Figure 11. The marginal contour in its limiting configuration is indicated by *m–m;* a collar isochrone, $C_t$ is drawn through the apexes of barbs $a_1u_1$ and $a_2u_2$. The difference in length of these two barbs approximates as a limit the length of shaft lying between the points of union of these barbs with the shaft. In text Figure 11, $a_1o = a_2o$. This is, of course, the same relation which we have previously described as the limiting relation between two simultaneously formed barbs in the limiting apical configuration.

If the ventral region of the germ can produce but one ridge (in each collar limb) at a time, the contour formation shown in Plate III could not be effected. Lillie and Juhn, however, have observed that the saddle-feather germ is characterized by a considerably greater width of tissue in the ventral region than is, for example, the germ of the breast feather (*v–v*, text Fig. 8). This ventral region bears no apparent ridges. It seems reasonable to believe that it is this undifferentiated ventral field of the germ which, under the influence of thyroxin, differentiates rapidly into definitive barb ridges. If this is true, the parallel between the limiting apical formation and the limiting contour formation induced by thyroxin in saddle germs is complete in both germ and regenerated feather.

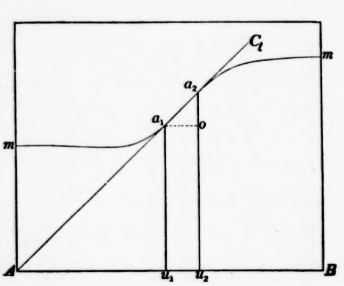

FIG. 11.—The geometric relations involved in the limiting subapical marginal contour, *m–m*. *A*, apical level of the section; *B*, basal level. *AB*, line of barb-shaft union; $a_1u_1$ and $a_2u_2$, barbs at right angles to *AB*. $C_t$ is the c-isochrone tangent to the limiting marginal contour; the limiting relation is that $a_1o$ shall be equal to $a_2o$.

We have found the limiting slope of marginal contours in only a few feathers treated with thyroxin, and all of these are saddle feathers which have been subjected to heavy dosages of thyroxin during regeneration. In most instances the marginal contour does not reach the limiting configuration shown in connection with the feather reproduced as Figure 8 of Plate III. But equally significant is the fact that we have found no instance in which barb apexes form a marginal contour with a slope greater than that of the c-isochrone.

In order to make evident the relations associated with formation of the limiting marginal contour at subapical levels, we present graphically, text Figure 12, several important relations. The curve *m*, text Figure 12*A*, represents the marginal contour, right vane-half, of the feather shown in Plate III. The contour of an approximate control is indicated by the light dotted line, *m*'. $C_t$ is a c-isochrone drawn through the marginal contour (*m*) at its region of greatest slope, *t;* ordinates, left.

Curve $f_m$ of Figure 12*B* represents the number of ridges formed per millimeter of axial growth from the apex of the feather through the 55-mm. axial level of growth (methods: Juhn and Fraps, 1936). Curve $f_s$ is the number of ridges completed[9] per millimeter of axial growth on corresponding c-isochrones.

Curve *r* of text Figure 12*A* gives the number of ridges in the collar on successive c-isochrones; ordinates, right hand of figure. The rapidly increasing number of ridges in the collar, beginning approximately with c-isochrone level 20, and reaching a maximum with c-isochrone 24, is referable to the limiting contour tangent, lying also along c-isochrone 24. The actual number of ridges formed per millimeter of axial growth, curve $f_m$ of Figure 12*B*, is very largely cumulative in curve *r* of Figure 12*A*. This is evident in the fact that thyroxin has effected only a small increase in the number of ridges completed per mm. axial growth ($f_s$ of Fig. 12*B*).

The relations shown graphically in Figure 12 represent in all probability an extreme result. Ordinarily, we have found that the number of barbs simultaneously formed by the action of thyroxin is considerably less than in this instance; also, the number of barbs in the collar generally shows a much lesser increase, and tends to fall more rapidly after having reached a maximum value. Finally, the shaft barb frequency ($f_s$, Fig. 12*B*) usually shows, in our experience, a much greater relative increase than does this example (cf. text Fig. 16).

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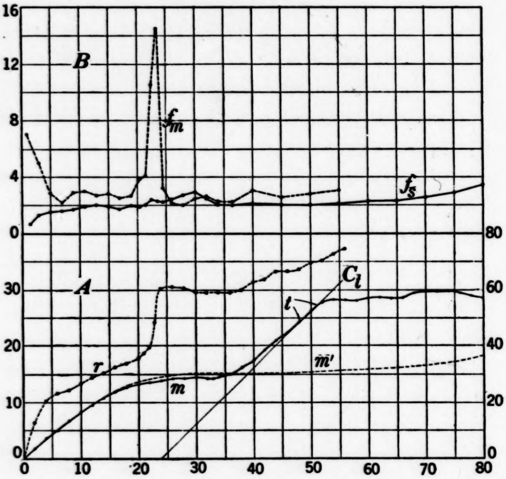
[9] The terms "ridges formed" and "ridges completed" are precise in meaning only with respect to actually defined loci in the regenerated feather as these are determined by c-isochrone constructions. We are not justified in supposing that definitive primordia exist in exact correspondence with these "loci" at the level of growth by cell division in the germ.

The important conclusion to be drawn from the relations presented in connection with text Figure 12 is that certain contour configurations, experimentally induced, approximate *as a limit* the c-isochrone co-ordinates which we have shown previously to be the limiting value also of tangents at the apex.



FIGS. 12*A* AND *B*.—Action of thyroxin, saddle feather, Brown Leghorn capon, reproduced as Figure 8, Plate III. Right vane-half only. All curves are referable to the single axis of abscissas, millimeters from apex. (Control feather, Fig. 9, Pl. IV.)

Figure 12*A:* $m$, the marginal contour of the modified feather; $m'$, the marginal contour of a control. Ordinates for contours are to the left of the figure and give barb lengths in millimeters. $C_t$ is the collar isochrone passing through the apexes of barbs formed simultaneously in the region $t$ of the contour curve, $m$. Curve $r$ represents the number of barbs in the collar; ordinates to right of the figure.

Figure 12*B:* the number of barbs formed per millimeter of axial growth ($f_m$) and the number of barbs completed per millimeter of axial growth ($f_s$).

## III. PIGMENTATION ISOCHRONES

The pigmentation isochrone (or p-isochrone) is the locus of points along which simultaneous pigmentation of the collar complement occurs, or can occur. The locus of simultaneous pigmentation can be located, at least approximately, with respect to definite structures of the germ, and thus affords a measure of certain general relations which cannot be otherwise defined.

We are interested here mainly in establishing the germinal relations which correspond with the deflection of p-isochrones from c-isochrones in the completely regenerated feather. A second point is to account for the observed differential rate of axial displacement of p-isochrones recorded by Lillie and Juhn (1932).

In general, p-isochrones, or the limiting boundaries of p-isochrones, do not coincide with c-isochrones. We have observed that p-isochrone boundaries may coincide with c-isochrones; this is true, however, only at or near the apex of the feather, and is apparently associated with the formation of simultaneous collar complements. Thus, in Figure 7 of Plate II, one boundary at least of the pigmentation isochrone, that lying along $p_1$, is practically identical in configuration with the c-isochrone. There is, however, a rapid increase in the slope of this boundary with successive periodic pigmentation formations of the marginal series ($p_2$, $p_3$).

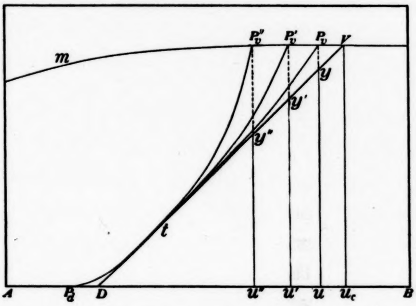


FIG. 13.—Diagrammatic representation of p-isochrone relations found in differing feathers. *AB*, the line of barb-shaft union; *m*, the marginal contour. The *p*-isochrones, $P_vP_d$, $P_v'P_d$, $P_v''P_d$, are drawn tangent to the c-isochrone, *VD*, at *t*. The ventralmost barb in each of these formations is indicated (relatively) by the section $yP_v$, $y'P_v'$, $y''P_v''$. Compare with text Figure 14.

We may assume that the limiting tangent drawn to p-isochrone boundaries is the c-isochrone. In general, however, the p-isochrone is deflected from the c-isochrone in the regenerated feather. The relations with which we have to deal are shown in text Figure 13. $P_vP_d$, $P_v'P_d$, and $P_v''P_d$ represent a series of pigmentation isochrones. These are so constructed in Figure 13 that the c-isochrone, *VD*, is tangent to each p-isochrone at *t*. The grounds for this construction are that the level of pigmentation occurs at or near the apicalmost limit of cell division in the germ. In any event, *VD* represents a c-isochrone parallel with reference to which differing p-isochrone curvatures can be compared.

Two characteristics are to be observed in the deflection of p-isochrones. These are:

1. With increasing deflection of the p-isochrone from the arbitrarily constructed c-isochrone there is an increasing length of barb lying between the marginal contour and the point of intersection of the basalmost barb to be pigmented. These are the segments $P_v y$, $P'_v y'$, and $P''_v y''$ of text Figure 13.

2. Corresponding with the increasing deflection of p-isochrones from the tangent c-isochrone, *VD*, there are increasing numbers of barbs between the marginalmost barb on the p-isochrone and the marginalmost barb on the c-isochrone. These are those barbs the apexes of which fall between *V* and $P_v$, $P'_v$, $P''_v$.

The relations in the germ which correspond with the observed order of deflection of p-isochrones from c-isochrones in the regenerated feather can be made clear by reference to text Figure 14. We are interested mainly in the ventral region of the regenerating germ, since it is here that the deflection of p-isochrones from c-isochrones is at a maximum. The observable relations in a germ in process of simultaneous pigmentation are: (*a*) The ventralmost ridge which is pigmented at its apex, $P_v$, text Figure 14, represents generally the maximum distance from the visible locus of ridge bases, $R_v R_w$. (*b*) There are visible in germs, generally, in process of simultaneous pigmentation, a number of unpigmented barb apexes ventral to $P_v$ of Figure 14. In addition to the visible number of ridge apexes, there are probably a number of "prospective" ridge apexes lying between *v–v* and the actual level of ridge origin.

We have observed also that there is a more or less evident correspondence between the degree of deflection of p-isochrones from c-isochrones in the regenerated feather and the degree of displacement (or slope) of the locus of simultaneous pigmentation of ventral regions from either the base of the germ ($C_v C_w$ of text Fig. 14) or the *visible* locus of ridge bases ($R_v R_w$, text Fig. 14).

The evidence of the preceding section requires that simultaneous axial growth increments (by cell division) be uniform through all regions of the collar. This conclusion is assumed throughout in the following description of p-isochrone formations.

### A. CONFIGURATION OF P-ISOCHRONES

The induction of simultaneous pigmentation reaction completely across the base of the feather germ is described by Lillie and Juhn (1932). A typical p-isochrone configuration induced by female hormone in a breast feather of the Brown Leghorn is shown in Figure 10, Plate IV. The locus of known simultaneous reaction is the anterior boundary of separation, *p–p*, between the normal black and the induced "female salmon" of the transverse bar. It will be noted that the line of isochrone pigmentation defines rather closely "the line approximately at right angles to the rhachis," which Lillie and Juhn observed to be characteristic of simultaneously induced pattern configurations.

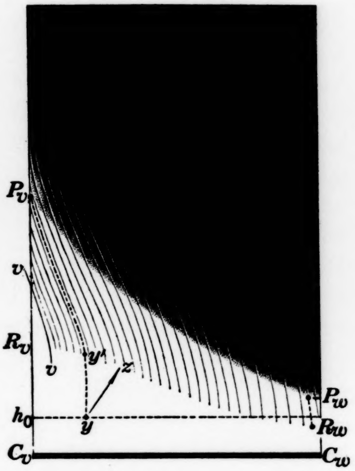


FIG. 14.—Diagrammatic representation of the ventral region of a regenerating feather germ, showing the relations involved in p-isochrone formation. $C_vC_w$, the base line of growth; $h_o$, c-isochrone parallel tangent to p-isochrones (cf. Fig. 13). The locus of simultaneous pigmentation is $P_vP_w$; the locus of apparent ridge bases falls along $R_vR_w$. The ventral triangle region is to the left of *v–v*. The ventralmost ridge to be pigmented is $P_vy'$ (cf. Fig. 13). Ridges to the left of this are without pigmentation in the germ. The assumed vector of growth by cell division is represented by *yz*.

We shall consider here, as examples of simultaneously imposed pigmentation configurations, experimentally induced patterns in the Brown Leghorn and the marginal pattern characteristic of the guinea-fowl secondaries. The experimentally induced p-isochrones (effected by female hormone and thyroxin) are controls from series studied previously by Lillie and Juhn (1932) in their analysis of simultaneous reaction in the germ. The p-isochrone configuration of the regenerated controls are of particular significance in view of the care exercised by Lillie and Juhn in establishing the conditions for induction of simultaneous reaction completely across the base of the regenerating germ.

### 1. P-ISOCHRONES INDUCED BY FEMALE HORMONE

The feather reproduced as Figure 10 of Plate IV is shown in Figure 11 with barbs mounted at right angles to the shaft. As basis for comparison of barb-shaft co-ordinates in c- and p-isochrones we have drawn paired c-isochrones symmetrically into the vane-halves of the feather; the symmetrically imposed c-isochrones are so located that the c-isochrone is tangent to the most basal of the p-isochrone formations in opposite vane-halves.

The barb-shaft co-ordinates of the p-isochrone in the right vane-half of a feather very similar to that reproduced as Figure 11, Plate IV, are shown in text Figure 15. Points of incidence of pigmentation on barbs from shaft to margin are connected by the line $P_vP_d$. The c-isochrone, $VD$, is drawn tangent to the p-isochrone at $t$.

It is clear that no c-isochrone can be drawn parallel to the p-isochrone, and further, that the p-isochrone is deflected from the c-isochrone increasingly, but not altogether regularly (note particularly the region *ab*, text Fig. 15), from shaft to margin. From shaft to margin in the regenerated feather, corresponds, of course, to dorsoventral in the germ. The portion of the p-isochrone from $c$ to $P_v$ (text Fig. 15), particularly, should then be found at a higher level above the theoretical region of embryonic growth by cell division at or near the ventral region in the germ than at intermediate or dorsal levels.

If, in text Figures 2 and 14, $h_0$ represents a parallel to, or within, the zone of growth by cell division, the locus of pigmentation reaction must be approximately of the form $P_vP_d$ in order to give us the general relation found in the regenerated feather (text Fig. 15). The relations here described are thus in agreement with the conclusion of Lillie and Juhn that the locus of pigmentation is at a higher level above the collar in ventral regions of the germ.

There can be little doubt that the zone of embryonic growth by cell division lies basal to the locus of simultaneously induced female hormone pigmentation (Lillie and Juhn, 1932). In terms of this assumed minimal relation between the c- and p-isochrone, there are a (minimal) number of barb apexes which lie on the c-isochrone but not on the p-isochrone (Lillie and Juhn). In text Fig. 15, these are the apexes lying between $P_v$ and $V$; in the germ, text Fig. 14, the corresponding (visible) apexes lie between $P_v$ and $v$–$v$. On this point, then, the relation between c- and p-isochrones in the regenerated feather are in complete agreement with the observations of Lillie and Juhn.

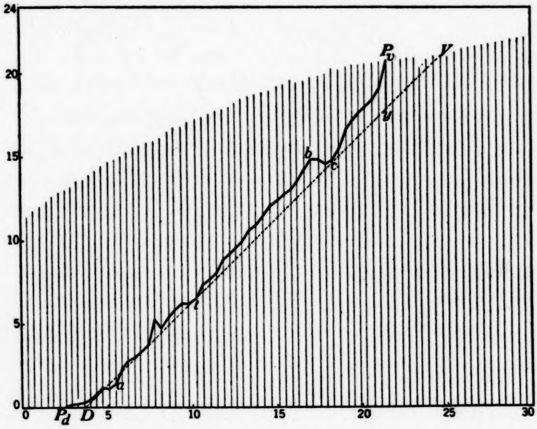


FIG. 15.—Experimentally induced p-isochrone in the right vane-half of a breast feather (female hormone induction). Abscissas, distances in millimeters from anterior limit of the feather section; ordinates, corresponding scale in millimeters. $P_vP_d$ is the locus of simultaneous pigmentation; $VD$ is the c-isochrone drawn tangent to $P_vP_d$ at $t$. $P_vy$, barb segment corresponding to maximum level of pigmentation above the collar. Ridges appearing unpigmented in the germ lie between $P_v$ and $V$. Compare text Figure 14; also Plate IV, Figures 10 and 11.

A second important observation, due also to Lillie and Juhn, is that short lengths of formed ridges at the ventralmost region of breast feathers appear entirely unpigmented. The corresponding barb segments in the regenerated feather are the marginal (barb) lengths lying between $P_v$ and $V$, text Figure 15, and marginal to the c-isochrone, $VD$. No ridge is pigmented in the germ in ventrodorsal order until it reaches a certain minimal length, which in text

Figure 15 is proportional to the barb segment $P_v y$, and which in any event cannot be less for this particular configuration (cf. text Fig. 14 for relations in the germ). Absolute distances in the germ are, of course, much less than in the regenerated feather, since the second phase of growth occurs later in time, and at a higher level, than does the pigmentation reaction.

The general relation between p-isochrones and c-isochrones shown in text Figure 15 are found to hold generally for experimentally induced female hormone patterns in breast feathers of the Brown Leghorn capon. There is, however, considerable variation in the exact co-ordinates of p-isochrones, particularly in degree of deflection shown by p-isochrones in different feathers at marginal limits (ventral in the germ) and in the asymmetry of p-isochrones in opposite vane-halves. We need not go into these relations here.

We have pointed out previously that it is at least theoretically possible that the axial rate of growth of ridges in the ventral region of the germ is more rapid than in dorsal or medial regions, and that we have not yet been able to measure the differentials involved. On these grounds the higher level of pigmentation in the ventral regions of the germ may be referable in part, but only in small part, to a higher rate of axial growth by cell division in these regions.

### 2. P-ISOCHRONES INDUCED BY THYROXIN

In at least some instances, the p-isochrones induced in saddle and hackle feathers by subcutaneous injection of thyroxin approximate more closely in angular configuration the theoretical c-isochrone than do similar isochrones of any type which we have thus far examined. Whether the slight degree of deflection shown by the p-isochrone in the feather reproduced as Figure 8 of Plate III, for example, is due to specificity of the reacting feather follicle or to some peculiarity of thyroxin action is not altogether clear. As is the case also with the female hormone formations discussed above, p-isochrones imposed by thyroxin show considerable variations (cf. text Fig. 16 with Fig. 8 of Pl. III).

The relations between c- and p-isochrones obtaining at the marginal region particularly are identical in order with those described above for p-isochrones induced by female hormone. Differences are concerned mainly with the less abrupt deflection of the thyroxin p-isochrone near the margin. Corresponding with the smaller deflection of thyroxin p-isochrones from the tangentially drawn c-isochrone (text Fig. 16), there are generally fewer barb apexes lying between $P_v$ and $V$. The marginal segment, $P_vy$, is likewise shorter, as a rule, than the comparable segment in p-isochrones induced by female hormone.

The typical configuration of the p-isochrone is independent of the spacing of barbs on the shaft. In some instances (e.g., Fig. 8, Pl. III) the spacing of barbs is altered but little by the action of thyroxin (text Fig. 12; in this and all similar figures in this paper, $f_s$, the number of barbs per millimeter of shaft length, is the reciprocal of the number of millimeters separating barbs at the shaft.) In text Figure 16 the spacing of barbs (curve $f_s$) is greatly altered, and the changes occur very rapidly. Moreover, the value of $f_s$ is changed in both directions from its normal value in this feather ($f_s$ is around 1.90 in comparable regions of control feathers).

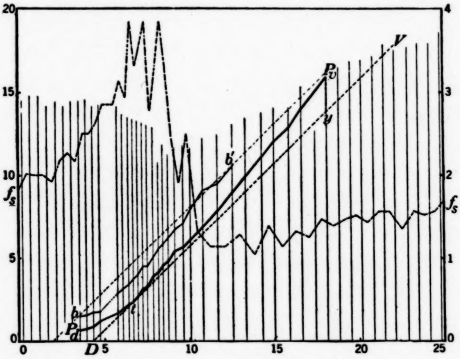


FIG. 16.—Configurations induced by the action of thyroxin in a neck hackle feather (see also Pl. III, Fig. 8); abscissas, distances in millimeters from apical end of the section; ordinates, left, corresponding scale in millimeters. $P_vP_d$, locus of simultaneous pigmentation; $b$–$b'$, locus of barbulation; $VD$, c-isochrone drawn tangent to $P_vP_d$ at $t$. The broken curve, $f_s$–$f_s$, is the curve of barb frequencies, ordinates at right of the figure. Segment of barb corresponding to maximum level of pigmentation at ventral region of the germ is indicated by $P_vy$.

Corresponding with changes in the spacing of barbs are changes in length of all barbs which were in the collar at the time when thyroxin effected pigmentation. The *absolute* value of the barb-shaft co-ordinates of the different p-isochrones are accordingly different in different instances. If the general form of the p-isochrones remains independent of absolute barb-shaft co-ordinates, we have additional evidence for the conclusion that the *ratio* of barb and shaft co-ordinates of the c-isochrone is constant. If this were not so, the p-isochrones induced by thyroxin would show characteristic shifts at differing regions, and these shifts would be more or less dependent upon changes effected in barb frequencies.

We are, or course, limited here to a *general* relation, since p-isochrones induced by thyroxin are never exactly uniform in different feathers. We are certainly not prepared to say that extreme thyroxin effects may not be associated with modifications, real or apparent, in the constant barb-shaft co-ordinates of the c-isochrone (and thus also of p-isochrones). The subject requires further and exact analysis, and we shall consider it elsewhere.

### 3. THE PATTERN OF GUINEA-FOWL WING SECONDARIES

The most striking evidence which we have been able to find that the angular deflection of the p-isochrones from c-isochrones in the regenerated feather can be referred to differential levels of reaction in the germ is afforded by the rapidly growing wing secondaries of the guinea fowl. The pattern of the guinea has been described by Hardesty (1933), who considered that the theoretical p-isochrone passed through homologous levels of transverse rows of "spots." The transverse rows of spots are not, however, always of simultaneous origin, and this is particularly true in the case of the wing secondaries, which we consider here.

The marginal bar of these feathers frequently takes the form of a relatively extended bar (Fig. 12, Pl. V; and text Fig. 17); and this bar is apparently of simultaneous origin, or approximately simultaneous origin, in the germ. In the left collar limb of the split-germ preparation reproduced as Figure 13 of Plate V, the ventralmost region is in process of forming one of the white bars which will lie marginally in the completely regenerated feather. This ventral region is shown enlarged in Figure 14, Plate V. The portion of vane represented by Figure 12, Plate V, is the corresponding vane-half of the same secondary from the opposite side of the bird. The marginal bar of the regenerated feather appears in the germ as an unbroken formation, its boundaries are fairly well defined, and it is apparently completely in one or another phase of pigmentation at any given time. We assume, then, that these bars are of simultaneous origin in the germ.

We cannot treat the entire transverse row of formations as of simultaneous origin with the marginal bar, in view of a complete discontinuity in the series at the level of the feather in which the marginal formation shows its greatest deflection from the c-isochrones. This situation is made clear in text Figure 17. We assume that the white spot numbered 14 of the intermediate series (the series lying between axial and marginal series) was formed simultaneously with the marginal bar of the same number (Hardesty, 1933). If an intermediate spot (15, 16, . . . . 22) forms simultaneously with a marginal bar (15, 16, . . . . 22), pigmentation must have occurred at two widely separated marginobasal levels of *the same barb* in the sixteenth period. At the seventeenth period an apparent "duplication" of the intermediate formation has occurred, and we might assume that simultaneous formation of complete transverse series has been re-established. But the same situation arises again by the time the twentieth marginal bar has formed. These relations are of considerable interest, but we cannot go further with them in this place.

The line of simultaneous ventral pigmentation in the germ is approximately indicated by $P_vP_w$, Figure 14, Plate V. The approximate line of *apparent* ridge bases is $R_vR_w$; we cannot exclude the possibility that ridge bases are in process of formation at levels below this line, or that additional ridges are not likewise forming or formed in the ventralmost extent of the collar. The locus of growth by embryonic cell division is assumed to lie at approximately the base of the germ ($C_dC_vC_d$ of Fig. 13, Pl. V).

Corresponding relations in the completely regenerated feather are shown in text Figure 17. The c-isochrone, *VD*, is approximately comparable with the locus, $C_dC_vC_d$, of Figure 13, Plate V, or with some higher parallel, for example, $h_0$ of text Figures 2 and 14. Assuming that the c-isochrone of text Figure 17 is tangent to the basalmost limit of the white bar, $P_v t$, the relations between the number of barbs in the collar, the number of barbs on this segment of the p-isochrone, and the number of barbs lying between the ventralmost limits of p- and c-isochrones must be at least *minimally* comparable with the visible formations, Figure 14, Plate V.

Ridges which have been formed but which are not cut by the p-isochrone lie between $P_v$ and $y'$ (or $y$). The barb segment, $P_v y$, of text Figure 17 is to be compared with the ridge, $P_v y'$ (or $P_v y$), in

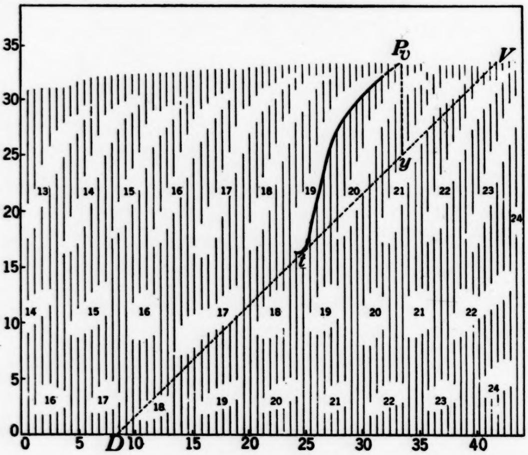


FIG. 17.—Section from a secondary of the guinea fowl wing. Abscissas, distances on shaft in millimeters; ordinates, corresponding millimeter scale. The pigmentation boundary, $P_v t$, is apparently of simultaneous formation in the germ; $VD$, c-isochrone constructed at about the level of $P_w$, Plate V. $P_v y$, relative maximum level of pigmentation in the germ; barb apexes between $P_v$ and $V$ are not pigmented in the germ at time of formation of $P_v t$. See text for description of pigmentation discontinuity between the three numbered series of formations.

the germ. It is clear that the level of pigmentation, $P_v P_w$, Figure 14 of Plate V, lies at a higher level with reference to the visible bases of ridges, $R_v R_w$, at or near the ventralmost limit of the germ than at other regions. The slope of the pigmentation locus with reference to the assumed locus of growth by cell division is, of course, even greater.

The number of barbs between the c-isochrone and the p-isochrone at the margin of the vane (the apexes between $P_v$ and $V$, text Fig. 17) is the least number of ridges which must have been formed or determined in the ventral region of the germ but which do not lie along the simultaneously imposed line of pigmentation reaction ($P_vP_w$, Fig. 14, Pl. V).

### B. THE DIFFERENTIAL GROWTH DISPLACEMENT OF P-ISOCHRONES

The p-isochrones first appear in the regenerating feather germ at differential levels from the visible line of barb bases and are probably even further displaced (differentially, in ventrodorsal order) from the assumed uniform c-isochrone. Growth from the base of the germ displaces the p-isochrone axially, and for a greater or lesser time the *rate* of axial displacement of ventralmost regions is greater than at medial or dorsal regions (Lillie and Juhn, 1932).

If we assume that there is no differential in the rate of cell division in the germ, or that such differentials are too small to account even for the initial differential locus at which pigmentation occurs, it is obvious that the observed more rapid rise of the p-isochrone in ventralmost regions must be referable to some other phase of growth. There are several possibilities which must be considered in this connection, and we shall simply call attention to these without attempting to evaluate quantitatively the observed differential displacements.

1. It is generally agreed that a second phase of growth, during which formed cells increase in size, accounts for the greater proportion of definitive length of the feather elements. It is necessary to assume that *total* growth of the second phase is a constant function of antedecent growth by cell division. The second phase of growth is, of course, initiated only after the conclusion of cell division.

Suppose that $h_1$ in text Figure 2 represents the level at which the second phase of growth is initiated *uniformly* in all elements of the feather germ, and assume also that growth in this phase is uniform at any given level above the base of the germ. The uniform axial displacement of the p-isochrone by cell division will bring ventralmost regions of the formed pigmentation line into this zone before regions dorsally are brought to the same level. Continued axial displacement of the p-isochrone by growth from the base of the germ will maintain ventralmost regions at higher levels if the *rate of growth* in the second phase is uniform. If rate of growth in the second phase is initiated gradually and increased to a maximum over a period of time (i.e., at higher levels), we should expect to find that ventralmost regions of a pigmentation line would continue to be displaced more rapidly from the base of the germ than are other levels of the same pigmentation line.

As regions of a p-isochrone dorsally removed from the ventralmost region are brought to higher levels within the zone of growth in the second phase, these also should show the accelerated displacement observed at an earlier time at the ventralmost segment of the same or similar p-isochrones. This is, in fact, the case. Finally, as the p-isochrone passes completely through the zone of growth by increasing cell size, we find that the initial displacement of the p-isochrone with reference to the base of the germ is gradually restored.

2. Growth in the second phase is not necessarily uniform in *rate* for all feather elements, even though the total enlargement effected in a cell bears a constant ratio to the original size of the cell. On theoretical grounds it might be expected that barb apexes, which are of smaller cross-section than basal regions of a barb, grow more rapidly during this second phase of growth than do basal levels of the barb. If this in fact occurs, the displacement of ventralmost regions will take place most rapidly from the base of the germ.

3. If we measure in the completely regenerated feather the length of barb segment between two identical p-isochrones, it is found that a greater length of barb lies between these two p-isochrones at their marginal limits, and that the length of this segment gradually decreases as we move toward the shaft. If the p-isochrone represented the primary locus of growth, the barb segments between two p-isochrones would become a measure of differential growth by cell division. If, however, the differential displacement of the p-isochrone represents a purely spatial displacement from the base of the germ at its moment of origin, the length of barb segment between parallel p-isochrones is referable to change in ventrodorsal *position* of the individual barb at successive p-isochrone reactions.

These considerations seem to account adequately for the observed differential rate at which p-isochrones are removed from their site of origin in the germ. There are at least no obvious grounds for believing that the differential axial displacement of p-isochrones following their origin must be referred to growth by cell division.

### C. DIFFERENTIALS IN P-ISOCHRONE REACTION

It was the general conclusion of Lillie and Juhn (1932) that differential pigmentation reactions are the result of rate differentials in the germ; the rate differential in which they were primarily interested was the axial growth-rate of individual barbs. But quite apart from the specific rate differential involved, Lillie and Juhn were careful to show that differentials did exist with respect to threshold of reaction and to the region in which pigmentation was effected. The objective evidence upon which Lillie and Juhn based their conclusions is that with increasing concentration of the reagents employed (female hormone and thyroxin) barbulation and pigmentation extends progressively from dorsalmost to ventralmost regions. Also, with high concentrations of female hormone effective for short times, pigmentation may be restricted to the ventral regions of the germ, and it appears first in the ventral regions in any event.

If the conclusions which we have reached in this paper are sound, differential axial rates of growth by cell division must be of a relatively small order, if they exist at all. That axial growth-rate differentials of an extremely small order may be the decisive factor in differential pigmentation reactions is certainly not disproved by our conclusions. It is not possible, on the other hand, to believe that the relatively great displacement of p-isochrones in the ventral region of the germ is evidence for the rate differentials in question.

We shall assume in this brief discussion of reaction differentials that axial growth by cell division is in fact simultaneously uniform through all elements of the collar. This may appear to involve a contradiction with the results of Lillie and Juhn. This is not the case, however. If it is realized that the lines of simultaneously uniform growth by cell division (the c-isochrones) occur always as a system of parallel lines in the vane-halves, then it must follow that any locus in the completely regenerated feather which is not parallel with the c-isochrone must involve differentials. The order of differentials involved is measured (if only geometrically) by the deflection between the two lines.

We may put this somewhat differently by saying that any reaction in the germ that is not parallel to the locus of the c-isochrone must involve differentials. If this were not so, all such reactions would compose, in the regenerated feather, a system of parallel lines. It is evident from the examination of simultaneously imposed pigmentation boundaries (text Figs. 15, 16, and 17) not only that the p-isochrones do not compose such a system of parallel lines but also that they show marked differences in different feathers.

Wholly without reference to the specific physiological nature of the differential involved, it is obvious that pigmentation isochrones represent differential reactions in the germ. *If the c-isochrone does in fact represent the locus of simultaneous growth in the feather germ, or is parallel to that locus, any reaction not parallel to the c-isochrone can only be understood to represent differentials (regardless of specific reaction or rate involved) with respect to the c-isochrone itself. Conversely, any isochrone configuration (pigmentation, barbulation) in the vane of a regenerated feather represents the projection of a differential reaction in the germ if that isochrone configuration is not parallel to the c-isochrone.*

We call attention to the fact that pigmentation isochrones are, under certain circumstances, practically parallel with the c-isochrone. The partial configuration, $p_1$, Figure 7 of Plate II, is an instance in point. These formations are undoubtedly associated with the formation of simultaneous collar complements; they are referable to a *uniform* initial displacement from the base line of growth, and also therefore, to uniform reaction differentials.

## IV. THE MECHANISM OF DEVELOPMENT

The experimental results (respecting growth particularly) and the conclusions based upon them have a direct bearing upon several aspects of development in the germ. These are, first, the nature of the primordia for shaft, barbs, and the ventral triangle region; second, the motions of growth; and third, developmental relations of a more general nature.

### A. PRIMORDIA OF THE FEATHER

We interpret the evidence from the constant fault configuration and the limiting contour tangents to mean that axial growth in the regenerating feather germ is uniform from ventral to dorsal regions If this is true, we can think of the embryonic base of the feather germ as an annular band of cells in process of growth by cell division. We recognize in this circumferential band (the collar of Lillie and Juhn) three segments which are distinctly differentiated after development of the germ is well under way. These are: (1) the shaft primordium which represents the dorsalmost region of the regenerating germ (text Figs. 7 and 8); (2) a ventral region which is undifferentiated but which, at least in certain instances, can be caused to undergo differentiation into ridges; and (3) the ridges of the bilateral collar complements lying between the ventral triangle and the shaft primordium.

Whether axial growth from the base of the regenerating germ is exactly uniform through the entire region of the collar ($C_vC_d$ of text Fig. 2) or subject to small differentials, it seems to us necessary to recognize the primordia described immediately above as differentiated regions or segments of the collar. This statement of germinal relations is at variance with the formulation given by Lillie and Juhn in 1932. These authors believed the primordium of the shaft to be coextensive with the annular base of the regenerating feather germ. The relations assumed to exist in the concrescence of the shaft primordia of opposite collar limbs are given diagrammatically in text Figure 18.

Text Figure 18 represents a section of a regenerating germ, including the shaft and a few barbs on each side of the shaft spread into the plane of the paper. The dorsally directed flow of the two halves of the shaft primordium, *R*, is indicated by the arrows. The semicircles, *e*, borne by each half of the shaft primordium, represent the growth centers of ridges, which, carried out of the zone of growth after union with the shaft proper, become increasingly separated (black disks), owing to increase in the axial dimension of the cells of the shaft. The points at which growth by cell division ceases are indicated by *u* for left and right halves of the shaft primordium; these points may be placed at a higher level without altering the primary relations postulated by the theory of concrescence.

We have pointed out that the c-isochrone relation requires that growth of the shaft be simultaneously identical with that of all barbs in the collar. The obvious difficulty in attempting to relate uniform *axial* increments to the theory of concrescence is that we must assume the *tangentially* directed growth of the shaft primordia to be exactly equal to the axial growth of the barb primordia from their individual growth centers, *e*. The difficulty of this interpretation becomes more apparent when it is realized that extension of the shaft primordium, which comes eventually to occupy practically the entire collar of the germ, would require that an increasingly massive shaft shall be produced by a constantly decreasing length of shaft primordium. This relation is clear by reference to text Figure 8; the shaft primordium, *S*, extends gradually toward the ventral limits, *v*, of the collar, thus gradually reducing the relative length of collar underlying the ridges.

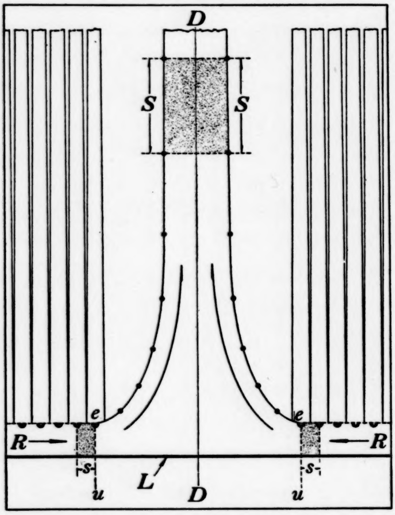


FIG. 18.—Diagrammatic representation of concrescence, based upon text Figure 1 of Lillie and Juhn (1932, p. 129). The central axis of the shaft is in the center of the figure (*D–D*); the dorsally directed primordia of the shaft are indicated by *R; L*, lip of the umbilicus. Distance between ridge primordia, *s;* distance between the same ridges after completion of growth, *S*. Growth centers of individual barbs, *e*.

The most decisive evidence which we have been able to obtain in favor of the limited extent of the shaft primordium comes from the relation between spacing of ridges in the collar and the spacing of barbs on the completed shaft. According to Lillie and Juhn, we should expect a constant ratio between the distance separating dorsalmost ridge centers in the collar and the distance separating barbs on the completed shaft (Lillie and Juhn, 1932).

Let *s*, text Figure 18, be the distance between the growth centers of the two dorsalmost ridges borne by a shaft primordium before concrescence. After completion of differentiation, the distance between these centers, *S*, is the distance between barbs on the shaft. According to the theory of concrescence $S=ks$; this is to say that the distance between barbs on the completed shaft is equal to the distance between the ridges which produce these barbs at their dorsalmost position multiplied by a constant, *k*, which represents the increasing size of cells in the second phase of growth.

It is a safe assumption, borne out by examination of germs, that the distance between ridge centers (*s* of text Fig. 18) increases with apicobasal formation of barbs through at least the period of growth occupied by the initial ridge complement. Following this period, the distances separating ridge bases in the collar may remain fairly uniform, but there is no evidence of decreasing distances between ridge centers in formation of the main vane.

In terms of concrescence, then, we should expect to find that the distance between these barbs on the definitive shaft increased in proportion to the increasing distance between ridge centers in the collar. We find, however, all manner of curves for the spacing of barbs on the shaft. Several of these are given in text Figure 19. Curve 1 gives the barb frequency (i.e., the reciprocal of distance between barbs) for a Brown Leghorn breast feather; curve 2 represents the barb frequency in a guinea breast feather; curve 3 shows the barb frequency in a hackle feather from a Brown Leghorn capon.

The order of increasing barb frequencies, i.e., decreasing distances between barbs, shown by curves 1 and 2, is clearly the opposite of what we should expect to find in terms of concrescence. If we assume that the ridges which join the shaft primordium from the apex to the fortieth millimeter of shaft growth are uniformly spaced in the collars which produced feathers represented by curves 1 and 2, it is necessary also to assume that these identical lengths of underlying shaft primordia are capable of producing lengths of definitive shaft varying by the values of the ordinates applying to these curves.

The spacing of barbs on the shaft is completely accounted for if we assume that the individual ridge may join the shaft at any time; it is only necessary to assume that axial growth in this given ridge and in the shaft primordium are uniform as long as the ridge remains in the collar. In our opinion, this identity of growth of ridge and shaft primordium can only be accounted for by the assumption that the shaft primordium represents a limited segment of the collar and is subject to the same growth-rates which at any moment obtain through the remainder of the collar.

If we conclude that the shaft primordium is not a bilateral, tangentially directed primordium in each collar limb, it follows also that the individual ridge growth centers postulated by the theory of concrescence cannot be passively carried from their site of origin at the ventral triangle to their point of union with the shaft proper. The alternative hypothesis of localized primordia for shaft and barbs and the growth relations by which we account for the ventro-dorsal translation of the barb primordia make it unlikely that the ridges have at their bases a fixed growth center. From this point of view it is probable that the growth and differentiation properties of the individual ridge are functions of their positions in the collar and

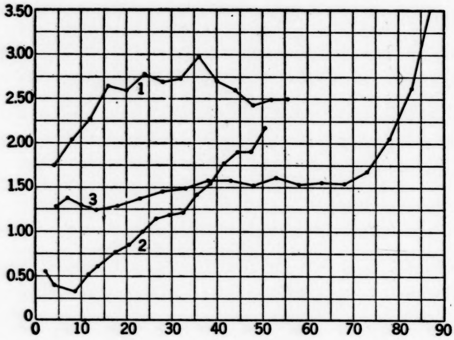


FIG. 19.—Barb frequencies at the shaft ($f_s$) in different feathers. Abscissas, distances in millimeters from apex of feathers; ordinates, number of barbs per millimeter of shaft length (or growth). Curve 1, left vane-half of breast feather, Brown Leghorn capon. Curve 2, breast feather of guinea fowl ♂ (Fig. 7 of Pl. II), averaged from both vane-halves. Curve 3, hackle feather, Brown Leghorn capon, $f_s$ averaged from both vane-halves.

that no fixed growth center need be postulated to account for the properties of the individual ridge.

### B. THE MOTIONS OF GROWTH

The statement of growth given in the introduction of this paper is purely descriptive. The relations involved may now be stated in somewhat more concrete form.

1. The results presented in this paper are evidence for the assumption of simultaneously uniform *axial* growth increments. This is simply to say that the entire base of the collar, including the shaft primordium, grows axially by uniform increments. We assume that the locus of axial growth lies at the base of the germ, $C_vC_d$ of text Figure 2 and $C_vC_w$ of text Figure 14. The grounds upon which we base the assumption of simultaneously uniform axial increments have been sufficiently emphasized and need not be described further here.

2. It is also necessary to assume the existence, in the regenerating feather germ, of a tangential component of growth. This tangential component accounts for the (apparent) motion of ridge bases from their site of origin to their site of union with the shaft. We may assume that the tangential component of growth is imposed at regions apical to the axially growing cells at the base of the germ, or that all cells in process of cell division are characterized by a vectorial displacement ($yz$, text Fig. 14).

If we assume that the axial component of growth is entirely independent of the tangential displacement, tangential growth must then occur at right angles to, and above the annular plane of axial growth at the base of the germ. Referring to text Figure 2, we may assume that the axial component of growth ceases at or about the level $h_0$. If the tangential component of growth is impressed at about this level, the definitive ridge structures will increase in size and be displaced from ventral to dorsal limits of the germ.

At the other extreme, we may assume that all growth by cell division in the feather germ represents a true vectorial displacement. This would be merely to assume that increasing numbers of cells are displaced, not strictly axially from the base of growth in the germ, but at an angle which would represent the resultant of increases in axial and tangential directions.

In any event, it is the tangentially directed vector of growth which accounts for the transposition of ridges from their site of origin at the surfaces of the ventral triangle to their site of union with the shaft. If the tangential vector is relatively increased, ridges will join the shaft more rapidly; if it is decreased relative to axial growth, ridges will join the shaft at a slower rate. The significant point is that tangentially directed growth of cells at the base of the germ, or within the definitive ridge structures, is adequate explanation for the ventrodorsal transposition of ridges.

One other point remains to be made clear: According to the conception of growth which we have set forth here, every transverse level of the completed barb arises at a definite ventrodorsal position in the collar. This can be made clear by reference to text Figure 2. Let us suppose that cells at $y$ represent the apical limit of the region of growth by cell division in the ridge $P_v y$. It is evident that the continuation of axial growth from the base of the germ must displace this level above $h_0$. At the same time, or independently, the tangential motion of growth displaces the point $y$ ventrodorsally. The locus of motion of the level $y$ is then along the line $yb$, text Figure 2. Similar relations may of course apply to the region of cell division below the visible bases of ridges. Thus, in text Figure 14 the level $y$ is displaced in the direction of the arrow to $z$. The apparent motion of barb bases becomes, in terms of the conception of growth given here, a function of vectorial displacements. Every level in the length of a barb is therefore referable to a definite position along the collar in ventrodorsal order. Stated conversely, the formation of successive transverse levels of the individual barb occurs at successive ventrodorsal positions in the collar.

### C. THE ORDER OF DEVELOPMENT

The developmental sequence of barb and shaft formation and the relations between them may now be summarized briefly as follows:

1. The first stage of differentiation of definitive feather elements is the formation of ridges in the wall of the collar. The order of ridge formation is dorsoventral with respect to the future site of shaft formation. The limiting "rate" of initial ridge formation is the simultaneous formation of a ridge complement. The marginal barb

frequency, i.e., the number of ridges laid down per millimeter of axial growth, is very high at the apex of the feather under these conditions. The two primary ridges appear to arise simultaneously in any event, and acute apical formations are referable to serial formation of ridges after formation of the two primary ridges.

2. Axial growth of the feather germ is initiated before formation of the shaft proper; this is evident in the fact that the two primary ridges grow through a greater or lesser length before they fuse at their bases to form the apex of the shaft (cf. Lillie and Juhn, 1932). The future site of shaft formation is determined at the time of origin of the two primary ridges (Greite, 1934). After the two primary ridges fuse at their bases, the single structure thus formed grows for a greater or lesser extent before ridges from the opposite collar limbs fuse serially with it. The spacing of barbs below the point of fusion of the two primary barbs is highly variable in different types of feathers, and is referable to growth relations involving axial and tangential rates.

3. With continuing development of the regenerating feather, the shaft primordium occupies an increasing tangential segment or arc of the collar. Toward the base of the feather, the segment occupied by the shaft primordium increases rapidly in extent, and during formation of the quill the entire annular base of the germ is projected axially as a cylindrical structure.

4. Following the formation of apicalmost ridges of the feather, ridges are laid down serially from a mass of undifferentiated tissue which becomes the ventral triangle of the germ. The ventral region is of variable extent in different feathers; that it may be of considerable extent is evident in the order of ridge formation effected by thyroxin.

5. Uniform axial growth increments obtain from formation of the apex of the primary ridges to the completion of regeneration in all feathers.

6. After the collar of the germ is definitely organized, it is subject to reaction differentials of the order described by Lillie and Juhn. These ventrodorsal differentials are evident in the deflection of lines of simultaneous pigmentation from the uniform locus of axial growth by cell division.

## V. SUMMARY

1. Evidence is presented for the conclusion that axial growth increments in the regenerating feather germ are uniform, or approximately uniform, for all elements in process of growth during any time interval. This conclusion is based upon an analysis of fault bars, normally occuring formations at the apex of feathers, and experimentally induced formations at subapical levels.

2. The relation of simultaneously imposed pigmentation configurations to the base line of simultaneously uniform growth by cell division is described, and the geometric relations between the two loci of simultaneous reaction are formulated as a spatial differential, independent of axial growth-rate differentials referable to cell division.

3. Interpretation of configurations in the feather has required a general statement of the mechanism of regeneration. The primordia of the feather and the motions of growth during development are formulated in terms which account for the properties of the configurations described in this paper.

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# EXPLANATION OF PLATES

All figures are photographs and microphotographs.

## PLATE I

### FAULT-BAR CONFIGURATIONS

Typical fault bars in several types of feathers.

FIG. 1.—Main tail feather, Brown Leghorn capon. The faults are characterized by marked reduction, or total absence, of barbules. The fusion of barbs on fault 4, right vane-half, is due to failure of ridges to separate during differentiation.

FIG. 2.—Wing primary, Brown Leghorn capon. The faults occur as defects in barbules and are very limited in axial extent; in some instances slight incisions occur in the shaft. Faults are limited wholly to the right vane-half; primaries generally show marked asymmetry in frequency of faulting in opposite vane-halves.

FIG. 3.—Major covert of secondary, Brown Leghorn capon. Marginal portions of barbs have been removed by cutting along lines of fault before mounting the feather; barbs are then mounted at right angles with the shaft. The several diagonals are drawn to make an angle of 45° with the line of union of barbs and shaft. These diagonals therefore define points equidistant on shaft and all barbs from the point of union of barbs with the shaft. Fault 5 is discontinuous in the mid-region of the vane-half.

FIG. 4.—Experimentally induced fault (adrenalin), breast feather, Brown Leghorn capon. The fault occurs as a well-defined bending of barbs from the plane of the feather vane. The photograph does not show true barb-shaft coordinates, inasmuch as the feather has been inclined to show the bending of the barbs.

## PLATE II

### APICAL CONTOUR CONFIGURATIONS

Figures 5 and 7 are two times the enlargement of the feather shown in Figure 6. Apicalmost c-isochrones, o, are drawn in each instance from apexes of the primary barbs at angles of 45° with central axis of the shaft. C-isochrones 10 are drawn from shaft intercepts 10 mm. from apexes of primary barbs. The ventralmost barb in the complement of simultaneously formed barbs is indicated by *i* in each vane-half.

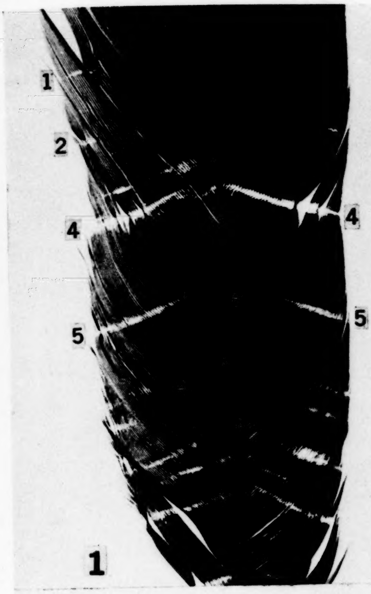
FIG. 5.—Breast feather from Barred Rock ♀; from or near the axis of symmetry of the tract. Unmounted barbs at the apex: left, 12; right, 11. Barbs formed simultaneously: 27 in the left vane-half, 22 in the right vane-half.

FIG. 6.—Hackle feather, Brown Leghorn capon, showing "acute" contour formation at its apex. This feather has been treated with thyroxin, repeated injections in low dosages. The form of the marginal contour is, however, typical of hackle feathers, and the pigmentation induced by thyroxin renders it especially suitable for reproduction.

FIG. 7.—Breast feather from guinea fowl ♂; position in the tract, on or near the axis of symmetry. Unmounted barbs at the apex, 4 on each side. Barbs formed simultaneously: left, 13; right, 11. The increasing displacement of pigmentation isochrones in apicobasal order of their formation is shown by slopes of the lines, $p_1$, $p_2$, $p_3$, drawn tangent to marginal "spots" of the pattern.

[See page 377 for legends for Plates III–V]

# PLATE I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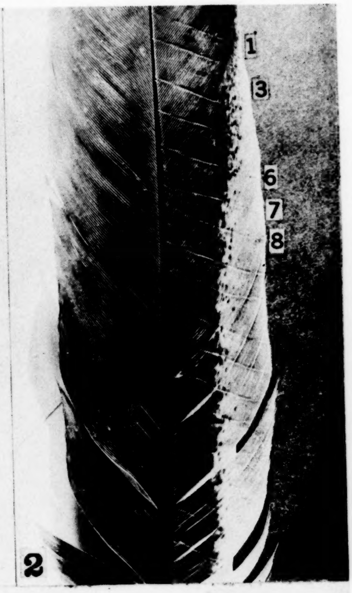
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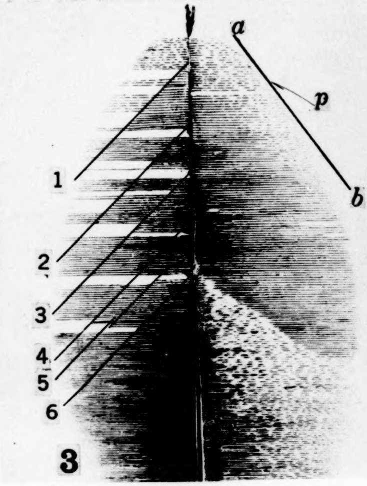
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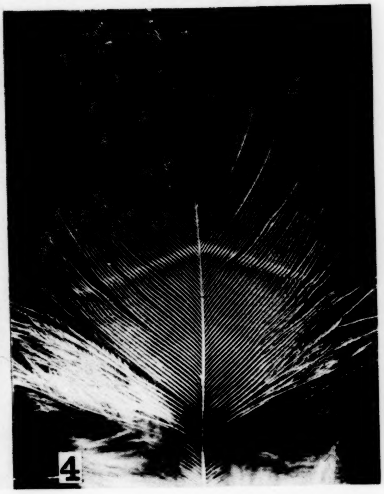
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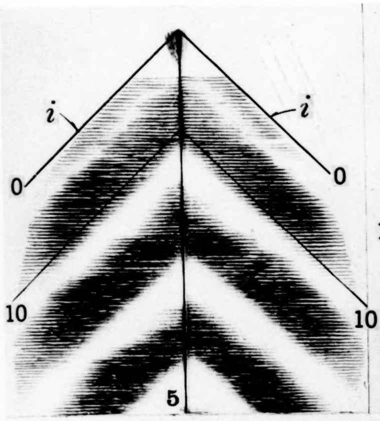
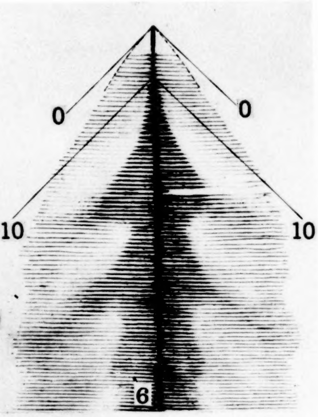
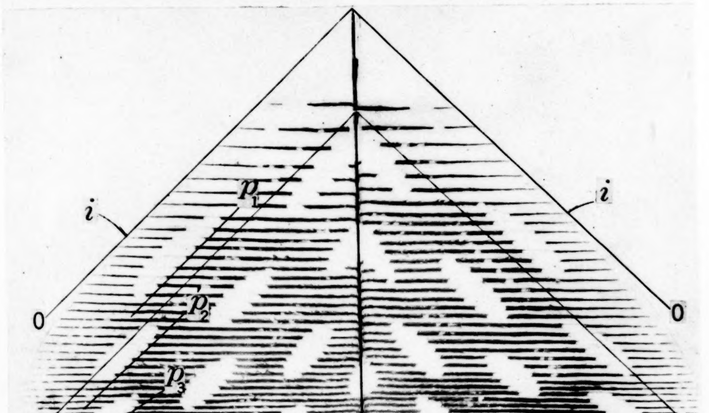
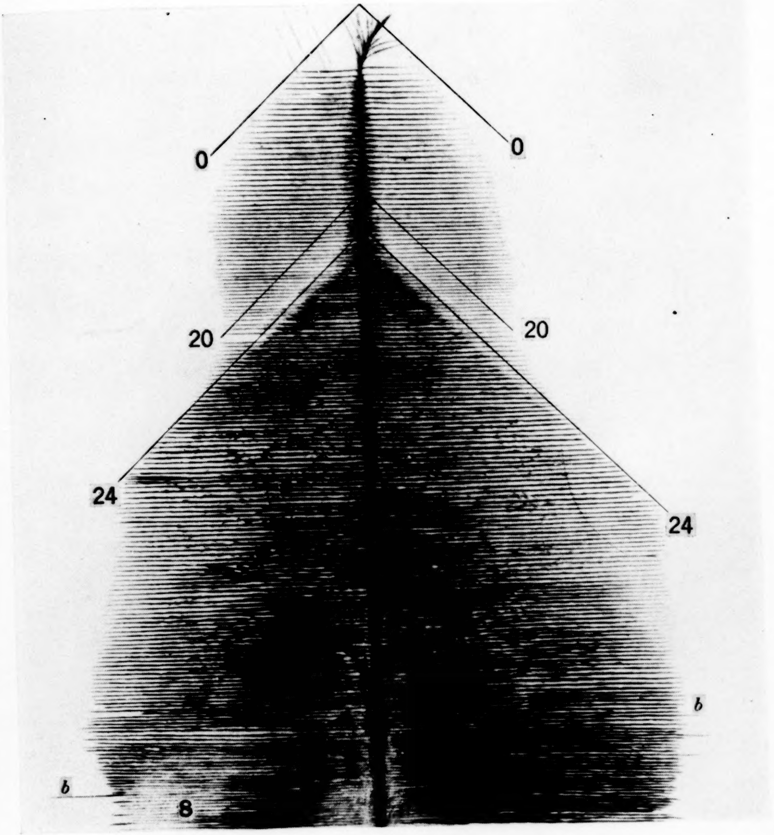


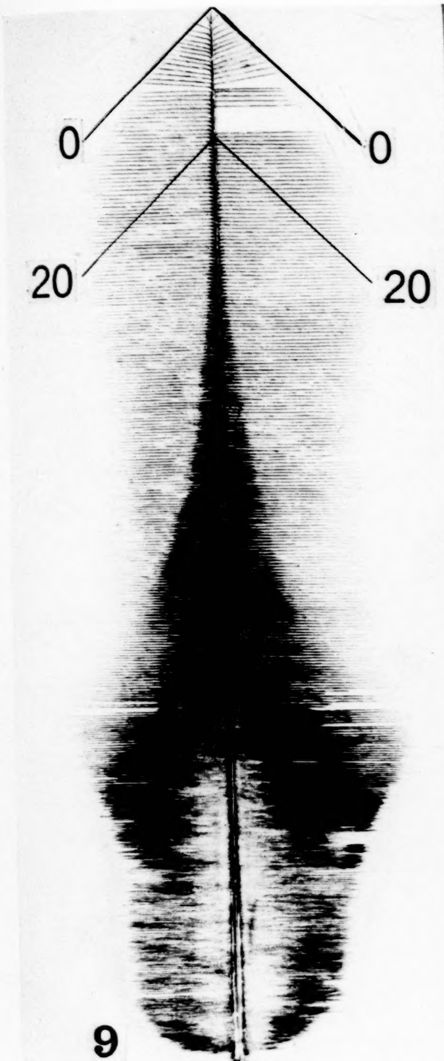



$p_1$
i
i
$p_2$
0
0
$p_3$
10
10
7

## PLATE III


0
0
20
20
24
24
b
b
8

# PLATE IV



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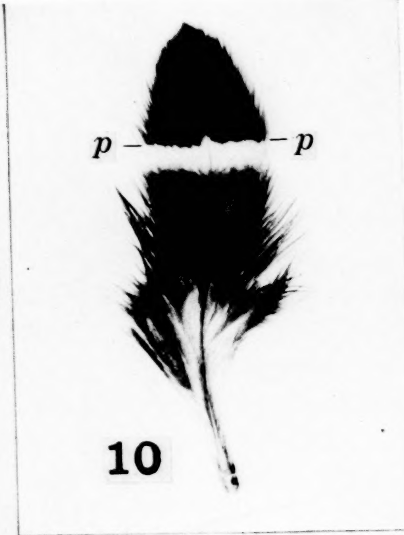
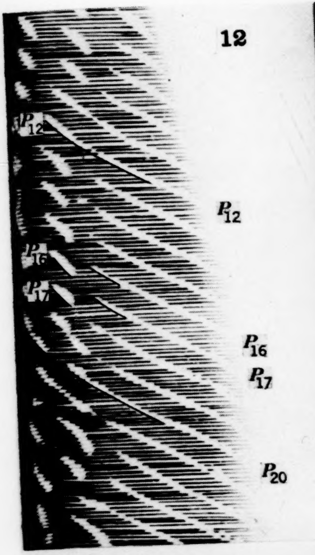
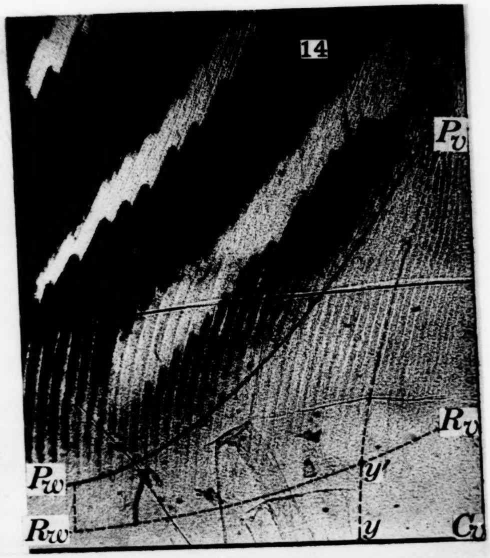


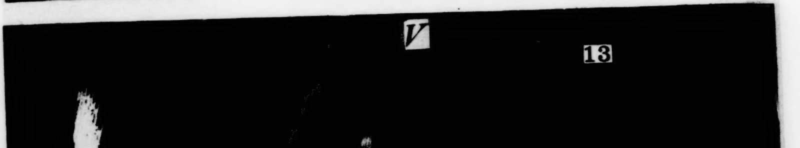
10

11

## PLATE V


12
P12
P12
P16
P17
P16
P17
P20


14
Pv
Rv
Pw
y'
Rw
y
Cv


V
13
l3
l2
l1
Pv
Pv
Cd
Cv
Cd

## PLATE III

### LIMITING MARGINAL CONTOUR IN MID-VANE REGION

FIG. 8.—Saddle feather, Brown Leghorn capon, single injection of 10 mg. thyroxin. Exact position unknown. (Approximate control shown as Fig. 9 of Pl. IV). The first evidence of thyroxin action occurs at c-isochrone 20 i.e., 20 mm. from the apex of the feather. The extension of the ventral triangle or the differentiation of this region has been completed at 24 mm. Barbs formed simultaneously, or approximately simultaneously, are clearly defined as those barbs, the apexes of which lie along c-isochrone 24 (cf. text Fig. 12). Occasional barbs in the "fluff" region extend considerably beyond the normal contours; two of these are indicated at *b*.

## PLATE IV

### PIGMENTATION ISOCHRONES: FIGS. 10 AND 11

FIG. 9.—Saddle feather, Brown Leghorn capon; approximate control for the saddle feather shown on Plate III.

FIG. 10.—Breast feather, Brown Leghorn capon, with experimentally induced female hormone pattern. The boundary of simultaneous pigmentation is the apicalmost boundary of the transverse bar, *p-p*.

FIG. 11.—The same feather shown in Figure 10, mounted with barbs at right angles to the shaft. The apicalmost boundary of the bar induced by female hormone deflects from the c-isochrone, $C_v$, in an apical direction; this is the characteristic deflection for simultaneously induced pigmentation lines in feathers of this type. Compare with the pigmentation line induced by thyroxin, Plate III.

## PLATE V

### RELATIONS AT THE VENTRAL TRIANGLE, GUINEA FOWL SECONDARY FLIGHTS

The three figures represent germs and portion of a secondary flight of the same position in the primary-secondary sequence.

FIG. 12.—Portion from the main vane showing the discontinuity of marginal and medial pigmentation formations. $P_{12}$ is continuous through the median formation. Discontinuity of the usual transverse formation occurs at $P_{16}$ and $P_{17}$ and is completed at $P_{20}$. We limit comparison of germs and regenerated feathers to the marginal pattern, corresponding with the ventral region of the germ.

FIG. 13.—Split preparation of the regenerating germ; the germ has been split along the center of the shaft, which therefore occupies the left and right limits of the photograph. $C_v$–$V$, the ventral superficial axis. $C_dC_vC_d$, the locus of growth by cell division. $P_v$ (left) and $P_v$ (right), ventralmost limits of pigmentation. The horizontals, $l_1$, $l_2$, and $l_3$, passed through centers of medial formations, show the increasing rate of growth of the second phase with increasing level above the base of the germ.

FIG. 14.—The ventral region from the left half of Figure 13, enlarged to show barb bases and the differential level of pigmentation with respect to barb bases. $P_vP_w$, locus of simultaneous pigmentation (corresponding to marginal bars of Fig. 12). $R_vR_w$, approximate locus of visible ridge bases. The base of the section is approximately along $C_dC_v$ of Figure 13. The ventralmost pigmented ridge is $P_vy'$. Apexes of unpigmented ridges fall between $P_v$ and $R_v$; their bases between $R_v$ and $y'$.

# DEVELOPMENTAL ANALYSIS IN PLUMAGE. III. FIELD FUNCTIONS IN THE BREAST TRACTS

(One plate and eight figures)

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THE degree of correspondence between vane-halves of the individual feather varies over a remarkable range. Certain feathers of all tracts are approximately symmetrical with respect to obvious characteristics, such as pigmentation patterns, the length of barbs, the distribution of barbs on the shaft, the distribution of barbules, etc. Other feathers from the same tract show asymmetry in some or all of these elements of structure or configurations of pattern. In some instances, e.g., the primaries, the asymmetry in the vane-halves becomes extreme and is evident in marked differences of pigmentation configurations, spacing of barbs, and mass of individual barbs. In other feathers, e.g., saddle feathers, the asymmetry is of much lesser apparent degree but is nevertheless easily recognizable in one or another respect in most feathers of the tract. These general relations have, of course, long been known and in some instances have received considerable attention from the morphological point of view (e.g., Chandler, 1916).

The distribution of differing symmetry types within plumage tracts has been treated quantitatively by Landauer (1930). Working with the spangled (apical) pattern of the Silver Spangled Hamburg, Landauer established general relations of reversed asymmetry on the two sides of the body. In at least one instance (the wing coverts of the male) he recorded a definite sequence of asymmetry types.

Lillie and Juhn (1932) considered the asymmetry of the individual feather in terms of physiological principles, i.e., in terms of

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[1] This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research; one of the authors (R. M. F.) was the recipient of a grant-in-aid from the National Research Council.

growth and development within the feather germ. Their analysis was based upon the discovery of Juhn and Gustavson (1930) that the threshold of reaction of the individual feather of the male and capon to female hormone is proportional to the axial growth-rate of the feather. Lillie and Juhn concluded that the asymmetry of the individual feather is a function of rates of growth in the two sides of the feather germ "at some time, or throughout development." Their interest was largely in the pigmentation configurations, and in certain definite instances they were able to observe asymmetries in the pigmentation pattern of the germ which were in close correspondence with configurations exhibited by the definitive feather. They also pointed out the dependence of degree of asymmetry upon position of the feather within the tract. Their observation of germinal differentials which were in correspondence with reverse asymmetries on opposite sides of the body are of particular interest. Lillie (1931) applied the principles set forth in the later paper by Lillie and Juhn to explanation of the partial and completely bilateral gynandromorphs found in birds.

Juhn and Fraps (1934) have noted that the symmetry relations along transverse rows of the breast tracts, as measured by lengths of barbs opposed at the shaft, followed an orderly distribution with respect to a secondary axis lying approximately at the sixth row, counting laterally from the mid-line of the bird. The increasing degree of asymmetry with reference to this secondary axis was in accord with previous observations of Juhn (unpublished data) that pigmentation patterns limited to one vane-half of the feathers of the breast tract showed the relation of mirror images within each tract, and that the degree of asymmetry in such patterns was of increasing order toward the lateral margins of the tract.

The study of Holmes (1935) demonstrated that there is a definite time and space order in the origin of follicles composing the individual plumage tracts. In the breast tracts particularly, the relation of this order to asymmetry in the adult plumage is clear: The first row of follicles to arise is parallel with the anteroposterior axis of the bird's body and is the sixth row of the definitive tract in lateral order from the mid-ventral line of the body. After the first row (No. 6 of the transverse sequence of the completed tract) has been laid down, anteroposterior rows lateral to this arise in order. Since the degree of asymmetry of the adult plumage increases with distance from the No. 6 row, we may conclude that the degree of asymmetry corresponds with the order of origin in time of the rows composing the tract.

On the foregoing grounds it seems probable that the asymmetry of the individual feather follicle is a function of its position in the plumage tract in a much more exact sense than has been shown thus far. If this is true, methods other than those used in previous analyses are required. We have considered methods in the first paper of this series (Juhn and Fraps, 1936), and in the present paper we apply several of the described procedures to further analysis of asymmetry in the breast tracts. Our results furnish additional evidence for reversal of asymmetry in transverse sequences of the breast tract at or near the sixth position in mediolateral order. We have been able to show also, however, that not all of the characteristics of the individual feather in these tracts are referable to this axis. These results are of considerable theoretical interest, and in the third part of this paper we discuss briefly the probable nature of embryological processes involved in determination of properties within the plumage tract.

## I. ORIGIN AND DISTRIBUTION OF FOLLICLES IN THE BREAST TRACTS

A brief statement of the observations of Holmes on the origin of follicles in the breast tracts will serve also to make clear the general arrangement of the individual follicles in the adult. The breast tracts are illustrated in text Figure 1 (from Holmes, 1935). *A–P* represents the anteroposterior axis of the bird. The row of black disks (1, 2, 3 . . . 26) represents the follicles of primary origin in the embryo, and is the sixth row in mediolateral order after completion of the tract. The follicles between the arrows are, according to Holmes, of approximately simultaneous origin in the embryo. Those anterior and posterior to the bases of the arrows arise later in time. After formation of the follicles composing the primary row, follicles arise to the right and left of it. The other rows composing the tract arise in order of distance from the row of origin in the tracts.

The individual follicles of the successive rows arise always at a point corresponding to the apex of an approximately isosceles triangle, the base of which is the line connecting two adjacent follicles of the previously formed anteroposterior row. The result of this order of origin is that the follicles of the completed tract represent a "herringbone" arrangement, and the lines connecting a series of follicles from the boundaries to the sixth row of the tracts are diagonals rather than right-angular co-ordinates.

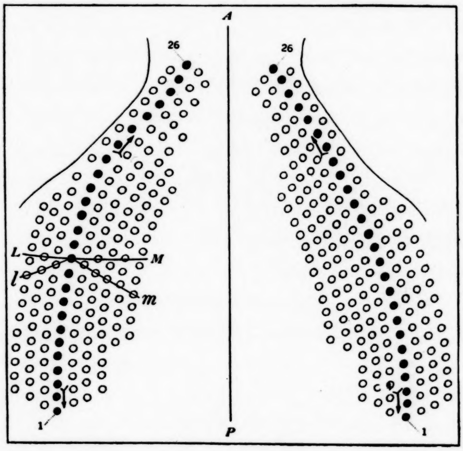

FIG. 1.—Order of origin of follicles in the breast tracts. *AP*, the anteroposterior axis (ventral) of the bird's body. The follicles represented as black disks and inclosed between the bases of arrows are of approximately simultaneous origin. Rows to right and left originate successively. The "true" transverse co-ordinate of the breast tract falls approximately along the line, *LM;* the actual transverse co-ordinate followed in experimentally ordered series is indicated by *lm*. (Adapted from Holmes, *Am. Jr. Anat.*, **56** [1935]: 513–37.)

In this paper we shall refer to a *transverse* sequence of follicles in the breast tract as the follicles along such diagonals, as *ml*, text Figure 1. The "true" transverse co-ordinate of the tract would lie along the line *ML*. We must take this displacement of lateralmost follicles from the true co-ordinates of the tract into account in the evaluation of certain results; this is particularly true of those properties of individual follicles which show also variation in anteroposterior order, e.g., axial growth-rates. Properties of individual follicles of the transverse sequence which show reversal at the No. 6 position are not, however, materially different along the true transverse co-ordinates and the diagonal co-ordinates actually used in practice.

## II. ORDER OF DISTRIBUTION OF PLUMAGE PROPERTIES IN THE TRANSVERSE SEQUENCE OF THE BREAST TRACTS

We are concerned here primarily with the distribution of properties in the breast tracts at approximately right angles to the anteroposterior axis of the tract (*ml* or *ML*, text Fig. 1). It should be clearly understood, however, that certain properties of the individual follicles (and feathers) of the tracts exhibit well-defined differences with respect to the anteroposterior axes. Juhn, Faulkner, and Gustavson (1931) have described at least two of these ordered functions: rate of growth of feathers and length to which regenerated feathers grow. Both these properties increase in anteroposterior direction. The fact that changes in rates and properties of the individual follicles vary through an area with two well-defined coordinates gives the breast tracts their primary *field* characteristics.

The properties of the nine follicles composing a transverse sequence of the breast tracts vary in three modes, which are conveniently distinguished as continuous, discontinuous, and polyphasic changes. A property which changes in the same direction from medial to lateral limits of the breast tracts, e.g., length of regenerated feather, will be referred to as a *continuous function* of the transverse co-ordinate of the tract. Properties which show definite reversal of direction of change (usually at or near the sixth follicle of the transverse sequence) will be denoted *discontinuous functions* of the transverse co-ordinates. *Polyphasic functions* describe properties which appear to involve both continuous and discontinuous functions along the transverse co-ordinate.

The follicles of the transverse sequence will be referred to always in mediolateral order as follicles 1 (nearest the ventral mid-line of the bird) to 9 (at the lateral boundary of the breast tracts).

### A. CONTINUOUS FUNCTIONS OF THE TRANSVERSE CO-ORDINATES

At least one property of the nine feather follicles composing a transverse sequence (*ml* of text Fig. 1) exhibits a continuous change in mediolateral order through the tract in the Brown Leghorn capon, to which the present discussion is limited. This is the length to which regenerated feathers grow. Curves representing the average length of regenerated feathers from two adjacent rows are shown in text Figure 2*B*. Mediolateral positions are indicated by 1–9 on the common abscissas of Figure 2 (*A* and *B*); average lengths of the regenerated feathers are given in millimeters. The continuous increase in length of feathers is unbroken through the region of the axis of symmetry of the tracts, position No. 6. The curves differ somewhat in left and right tracts, but the general relations are well defined and cover a sufficient range of differences to eliminate the possibility that lengths of feathers along the true transverse co-ordinate (*ML* of text Fig. 1) might show a different relation. Since the length of completely regenerated feathers increases on all rows in anteroposterior order, the length of feathers on rows lateral to the sixth row of our curves are, in fact, somewhat less on the true transverse co-ordinate. Taking No. 6 as fixed, the net effect of this correction would be to flatten the curves slightly from positions 6 to 9 and to steepen the limb from positions 1 to 6.

A second set of lengths are plotted in text Figure 8. The plotted values represent average length and weight of feathers from six transverse sequences. Positions in the mediolateral sequence are given as abscissas; lengths in millimeters, by the left-hand ordinate. The relation of length of feather to position on the transverse co-ordinate is very similar to that shown in the curves of text Figure 2*B*. Incidentally, we might call attention to the marked relation between average length and average weight of feathers from the No. 1 to No. 6 positions of the transverse sequence, in contrast with the definite change in this relation shown by feathers from the 6 to 9 positions.

The individual measurements upon which the curves of text Figure 8 are based are given in Table I, together with weights of the same feathers. The feathers from corresponding positions are arranged in the usual mediolateral order, 1–9. The several sequences are from three birds, Nos. 1, 2, and 3, in the table. Lengths of feathers (millimeters) on two transverse sequences are given under *a* and *p* for bird 1. The number of follicles separating the sequences is not recorded, but row *a* was probably considerably anterior to row *p*. The figures for birds 2 and 3 represent averages for adjacent feathers from the same transverse position. While there is some degree of variation in the order of changing length with position, the results are of much the same order. It is to be noted particularly that significant increases in length occur from sixth to ninth positions on all transverse sequences.

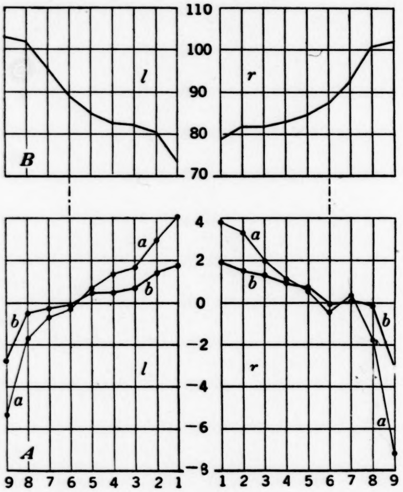

FIG. 2.—Graph *A:* differences in lengths of barbs in opposite vane-halves of feathers on 2 transverse sequences of the breast tracts. The figures at the base of the graph represent order of follicles from ventral mid-line of the bird's body. Ordinates, differences in millimeters, opposite vane-halves. Curves *a*, maximum differences in barb lengths; curves *b*, differences in barb lengths over the centimeters of greatest difference. Graph *B* gives length of the same feathers used in calculating the curves of graph *A;* ordinates, lengths in millimeters. Left and right sides of body: *l* and *r*.

The length of regenerated feathers is certainly not referable to the secondary axis of symmetry in the breast tracts of the Brown Leghorn capon. We have thus far been unable to be certain that any other property of the individual follicles shows so complete an independence of the axis of asymmetry (reversal at No. 6 position) as does this particular property. The length of a regenerated feather is a definite result of growth characteristics of the individual follicle (not necessarily growth-rates), and as such we conclude that it measures a regenerative function of position with reference to the ventral mid-line of the bird's body.

We shall show later that rate of growth is not entirely correlated in the transverse sequence with length to which the individual feather grows. Inasmuch as the total length of the regenerated feather must, however, represent growth, we need only refer this property to longer period of growth, differences in growth-rates, or to combinations of these which may or may not in themselves be referable to other axes of the tract.

TABLE I

WEIGHTS AND LENGTHS OF FEATHERS FROM TRANSVERSE BREAST SEQUENCES

| POSITION No. | BIRD 1: *a* | | BIRD 1: *p* | | BIRD 2: *a, b* | | BIRD 3: *a, b* | | AVERAGES | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mg. | Mm. | Mg. | Mm. | Mg. | Mm. | Mg. | Mm. | Mg. | Mm. |
| 1 | 41.4 | 75.0 | 39.5 | 83.5 | 28.3 | 77.0 | 35.9 | 75.3 | 34.9 | 77.2 |
| 2 | 40.5 | 84.0 | 39.4 | 84.0 | 30.9 | 77.0 | 37.6 | 76.8 | 36.1 | 79.2 |
| 3 | 39.7 | 85.0 | 41.4 | 85.0 | 32.5 | 78.5 | 38.3 | 76.5 | 37.1 | 80.0 |
| 4 | 41.2 | 85.5 | 40.6 | 87.0 | 34.0 | 79.0 | 40.0 | 79.8 | 38.3 | 81.7 |
| 5 | 43.5 | 89.5 | 42.0 | 91.5 | 37.3 | 83.5 | 41.9 | 82.3 | 40.6 | 85.4 |
| 6 | 44.9 | 91.5 | 46.5 | 94.0 | 41.6 | 84.5 | 45.1 | 87.0 | 44.1 | 88.1 |
| 7 | ...... | ...... | 44.1 | 95.0 | 41.4 | 93.8 | 47.8 | 93.8 | 44.5 | 94.0 |
| 8 | 47.7 | 94.0 | 45.0 | 100.0 | 41.4 | 99.0 | 48.0 | 99.8 | 45.3 | 96.9 |
| 9 | 44.5 | 94.0 | 45.2 | 101.0 | 40.0 | 97.8 | ...... | ...... | 42.4 | 97.6 |

Juhn, Faulkner, and Gustavson (1931) concluded that the rate of growth of regenerated feathers increased in mediolateral order from the ventral mid-line. Their measurements were made over relatively great intervals of time, and it is difficult to interpret their conclusions in terms of actual rates, and particularly rates which refer to more or less comparable periods in the regenerative cycle. A further analysis of growth-rates with reference to the transverse sequences of the breast tracts has shown that the maximum rate of growth must be defined for homologous phases in the regenerative cycle rather than as "averages" for relatively prolonged periods. These findings (unpublished data of Juhn) are probably of significance also in connection with the difficulties in interpretation of plumage response in breast tracts to intramuscular injection of oestrone recorded by Greenwood and Blyth (1935*a*). We take up these growth-rate relations in later pages.

### B. DISCONTINUOUS FUNCTIONS OF THE TRANSVERSE CO-ORDINATES

By "discontinuous functions" we refer to those properties of the individual follicle which show a definite reversal of asymmetry or sign at or near the No. 6 position of the transverse sequence. Such functions are "discontinuous" with respect to the mediolateral coordinate of the breast tracts, and "reversal" in any relation or property involving opposite vane-halves occurs as a change in sign if we relate magnitudes describing the vane-halves uniformly for all feathers of the transverse sequence.

#### 1. LENGTH OF OPPOSED BARBS IN BREAST FEATHERS

In a brief note by the present authors (Juhn and Fraps, 1934), we showed that *differences* in the length of simultaneously completed barbs in the series of breast feathers composing a transverse sequence were least at or near the No. 6 position, and that differences in length of opposed barbs increased in order of progression toward lateral margins of the tracts. These results have since been considerably extended. We give in text Figure 2*A* curves for two length indexes of barbs in opposite vane-halves. The numbers 1–9 from the center of the graph represent transverse positions in mediolateral order from the ventral mid-line of the bird; ordinates represent differences in length of barbs in millimeters. Curve *a* in each graph is the maximum difference in length of completed barbs; curve *b* represents average differences over the centimeter of greatest difference in length of simultaneously completed barbs. The two curves show that least differences are found in the neighborhood of the No. 6 axis. This is more definitely brought out in the left breast tract than in the right, but in neither instance is there any doubt concerning the general trend of the curves from medial to lateral margins of the tract.

It should be pointed out that differences in lengths of simultaneously completed barbs are plotted continuously across the tract; a value of 0, therefore, represents no difference in the two vane-halves of the feather and is an index of approximate symmetry with respect to the particular index chosen. Negative differences become positive, of course, if we reverse the order of subtraction in vane-halves at (or approximately at) the No. 6 position in mediolateral progression. The usual relation of reversed symmetry with reference to the No. 6 position is then evident by the upward curvature of both limbs of the curve from the No. 6 position.

In recording measurements of length of simultaneously completed barbs at intervals from apex to base of the shaft, we have observed that there is a common tendency for differences in lengths to show a definite order of change in at least those feathers near No. 6 positions. It is difficult to interpret in terms of asymmetry either maximum differences in barb lengths or averages of differences for any considerable apicobasal section of these "least asymmetric" feathers. This situation is made clear in text Figure 3(*A* and *B*), which shows the differences in length of opposed barbs at 5-mm. intervals from apex of the shaft through 80-mm. shaft lengths. The curves of Figure 3*A* are for feathers from the No. 6 positions; curves of Figure 3*B* are for feathers from the No. 7 positions. The letters *a* and *b* indicate adjacent anterior and posterior feathers.

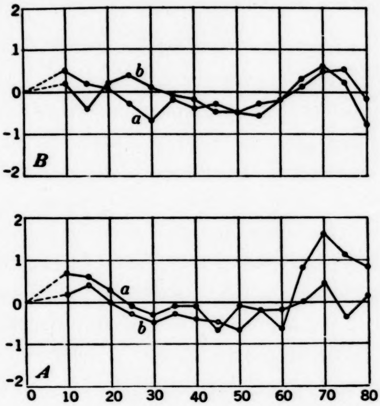

FIG. 3.—Reversal in lengths of barbs at completion in opposite vane-halves. Abscissas, distances from apex of feather in millimeters. Ordinates, differences in barb lengths in millimeters. Graph *A*, feathers from the No. 6 position; graph *B*, feathers from the No. 7 position. The small letters, *a* and *b*, refer to individual feathers from different anteroposterior sequences.

It will be observed that differences in barb lengths are positive through approximately 20 mm., after which differences become negative, and remain so, to about 60 mm. At or near the 60-mm. shaft level there occurs a second reversal of sign. It is probably significant that reversals of this order are more marked in feathers near the sixth position. While the asymmetry of an individual feather, as based upon an "average" index, may be close to zero, it is obvious that an index based upon differences in lengths of barbs at chosen intervals along the shaft may give either positive or negative values for the "asymmetry" of the feather. For these reasons we have considered the curves given in text Figure 2*A* to be simply "indexes," which, while indicating a real reversal of asymmetry at or near the No. 6 position, are not of great value in other respects.

### 2. ASYMMETRIC MAGNITUDES DETERMINED BY C-ISOCHRONE ANALYSIS

Methods for the application of the c-isochrone to the completely regenerated feather are described in the first paper of this series. We have applied these methods (in part) to three feathers of a single transverse sequence. The results given here are concerned mainly with determination of relations in the opposite vane-halves. The feathers used for analyses are treated individually, and we shall attempt to show only that the various determined magnitudes agree in locating the axis of reversal of asymmetry in the neighborhood of the sixth position of the transverse sequences. A complete analysis of the breast tracts of the Brown Leghorn will require treatment of more comprehensive series than we have yet attempted, and the present results are necessarily of a preliminary nature. It will nevertheless be clear that reversal of signs in magnitudes measuring relative asymmetry occurs definitely in feathers 3 and 9, while in feather 6 the same measures show least values. The quantitative formulation of similar relations for complete sequences will be the subject of a later analysis.

The three feathers for which we present data are from a Brown Leghorn capon. We have some evidence that asymmetry differentials are less marked in the female; whether these differences are due to individual variations rather than to differences in sex has not been determined. A few preliminary results on breast tracts of the Barred Rock show also rather marked deviations from the results given here. For these reasons we wish to make it clear that the conclusions drawn from this analysis are limited to the Brown Leghorn capon.

*a) Cumulative barb numbers.*—The derivation of cumulative barb numbers and their significance are taken up in the first paper of this series.

The marginal c-isochrone determinations for feathers 3, 6, and 9 of a transverse sequence (feathers reproduced in Pl. I) are given in Table II. Column *c* is the number of successive c-isochrones; *d* is the distance in millimeters from apex of the feather to successive c-isochrones; *l* and *r* refer to cumulative numbers of barb apexes defined in left and right vane-halves. It is evident from the figures presented in this table that feather 6 shows least differences in opposite vane-halves.

TABLE II

MARGINAL BARB NUMBERS, FEATHERS 3, 6, AND 9 OF TRANSVERSE BREAST SEQUENCE

| *c* | FEATHER 3 | | | FEATHER 6 | | | FEATHER 9 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *d* | *l* | *r* | *d* | *l* | *r* | *d* | *l* | *r* |
| 1...... | ....... | 25.0 | 15.0 | ....... | 8.5 | 7.2 | ....... | 3.0 | 11.2 |
| 2...... | 0.64 | 29.7 | 23.5 | 0.45 | 17.0 | 14.8 | 0.64 | 9.0 | 22.6 |
| 3...... | 1.28 | 32.7 | 29.2 | 1.12 | 28.0 | 25.0 | 1.57 | 19.4 | 33.5 |
| 4...... | 1.92 | 36.4 | 34.2 | 1.95 | 37.2 | 34.6 | 2.69 | 31.0 | 43.0 |
| 5...... | 2.88 | 41.8 | 42.7 | 3.36 | 47.6 | 45.2 | 4.32 | 44.0 | 51.0 |
| 6...... | 4.16 | 49.0 | 52.0 | 4.70 | 55.0 | 52.0 | 7.08 | 57.0 | 60.8 |
| 7...... | 5.77 | 56.6 | 59.0 | 5.82 | 60.3 | 57.0 | 9.79 | 69.7 | 71.8 |
| 8...... | 7.69 | 64.8 | 68.0 | 7.97 | 70.7 | 66.2 | 14.34 | 87.0 | 86.0 |
| 9...... | 10.24 | 75.0 | 77.2 | 10.88 | 81.8 | 77.0 | 18.01 | 100.2 | 99.0 |
| 10...... | 13.77 | 88.0 | 92.0 | 13.50 | 90.7 | 86.7 | 22.34 | 116.0 | 112.0 |
| 11...... | 17.92 | 101.2 | 107.0 | 16.38 | 100.0 | 95.6 | 27.42 | 131.0 | 126.0 |
| 12...... | 22.72 | 116.0 | 122.0 | 19.52 | 110.0 | 105.8 | 32.70 | 147.0 | 138.5 |
| 13...... | 27.74 | 128.0 | 135.0 | 23.42 | 121.5 | 118.0 | 39.68 | 166.0 | 156.0 |
| 14...... | 31.55 | ....... | ....... | 27.04 | 132.0 | 127.2 | ....... | ....... | ....... |
| 15...... | ....... | ....... | ....... | 30.82 | 143.0 | 138.0 | ....... | ....... | ....... |
| 16...... | ....... | ....... | ....... | 34.40 | 152.0 | 147.5 | ....... | ....... | ....... |
| 17...... | ....... | ....... | ....... | 37.44 | 160.0 | 156.0 | ....... | ....... | ....... |

The data of Table II applying to feather 3 are represented by the curves $N_m$ of text Figure 4; the corresponding data for feather 9 are given in text Figure 5. Abscissas in both figures represent distances in millimeters from apex of the feathers; marginal barb numbers, $N_m$, are represented by ordinates.

Marginal barb numbers define the cumulative numbers of barb apexes "determined" at given axial levels of growth (Juhn and Fraps, 1936). Similarly, shaft barb numbers define the number of barb bases "completed" at definite axial levels of growth. Shaft barb numbers are given in Table III, and the data are graphed as curves $N_s$ in text Figures 4 and 5 for corresponding marginal barb numbers and abscissas (feather 3 in Fig. 4; feather 9 in Fig. 5).

Marginal barb numbers increase with relatively great rapidity near the apex of both feathers. During the initial stages of barb formation, marginal numbers are higher in the left vane-half of feather 3 (text Fig. 4); in feather 9 (text Fig. 5) the reverse is true. In both feathers 3 and 9 the initial relations are subsequently reversed with respect to opposite vane-halves.

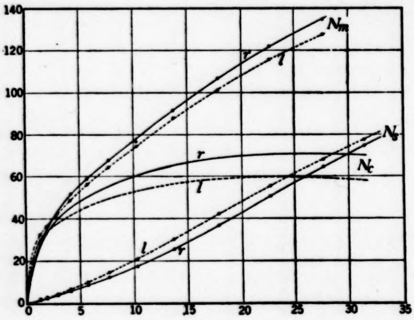

FIG. 4.—Asymmetry relations defined by c-isochrone constructions in feather 3 of transverse breast sequence (Pl. I). Abscissas, shaft distances in millimeters from apexes of the primary barbs; ordinates, barb numbers. Curves $N_m$, marginal barb numbers; curves $N_s$, shaft barb numbers; curves $N_c$, barbs on successive isochrones ($N_c = N_m - N_s$). Broken lines (*l*), left vane-half; solid lines (*r*), right vane-half.

These relations apparently introduce in a quite different form the reversal of asymmetry which we have already observed to be characteristic of difference in lengths of simultaneously completed barbs.

It is obvious that the initial stages of barb formation (curves $N_m$) represent relations which are the reverse of relations obtaining in formation of the main vane structure, i.e., from approximately 5 mm. basally. The initial phase of ridge formation is characterized by simultaneous, or very rapid, formation of ridges in proportion to axial growth of the germ (Fraps and Juhn, 1936). This initial rapid rate of barb formation represents the formation of ridges in *dorsoventral order* in cells already formed in the wall of the feather germ. After completion of collar-limb complements, barbs are laid down serially at the ventral triangle of the germ. The formation of ridges in these differing modes is probably associated with the *apparent* reversal of relations so clearly brought out in the curves of text Figures 4 and 5. If this is true, it is highly probable that both initial formation of ridges and serial formation of ridges through the main vane structure are referable to the same fundamental measure of asymmetry (cf. text Fig. 6).

The curves for shaft barb numbers, $N_s$, as measured by cumulative numbers of barb bases at successive c-isochrones, show similar relations. The *absolute* relations are, however, the reverse of those shown by curves for cumulative marginal barb numbers. This is clearly brought out if we compare the four curves beyond the first 7 mm. of shaft length. Cumulative shaft numbers (feather 3) are higher in the left vane-half; the corresponding marginal numbers are lower in this vane-half. The curves for feather 9, text Figure 5, show exactly the opposite relations in every respect beyond ca. 12 mm.

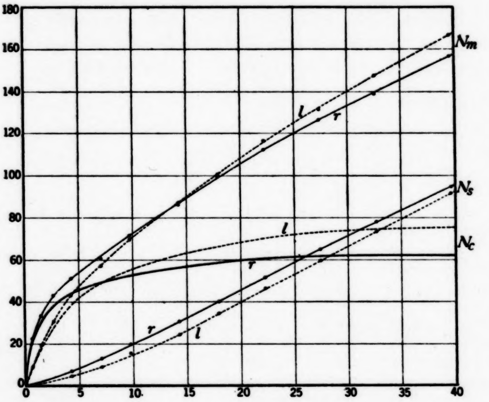

FIG. 5.—Asymmetry relations in feather 9 of transverse breast sequence. Abscissas, shaft distances in millimeters from apexes of primary barbs; ordinates, barb numbers. Curves $N_m$, marginal numbers; curves $N_s$, shaft numbers; curves $N_c$, barbs on successive c-isochrones. Broken lines (*l*), left vane-half; solid lines (*r*), right vane-half. Note the reversal of all relations shown in text Figure 4.

If we take some level following establishment of the complete barb complement, say at about the 30-mm. level, the left collar limb of feather 9 (text Fig. 5) is laying down barbs at a more rapid relative rate. These barbs are completed, however, at a slower relative rate. Again, feather 3 shows opposite or "reverse" relations in every detail.

The figures of Table II show that feather 6 is subject to relatively small differences in cumulative barb numbers at the margin (barbs laid down). Differences in relative numbers of barbs completed are even less, as is shown in Table III.

It is not necessary to attempt an interpretation of these results further than to point out that actual numbers of barb apexes laid down, or of barbs completed, represent definite "events" in the developmental process. If the numbers which measure these events show differentials in the two vane-halves of an individual feather, and a definite reversal of these differentials in feathers on opposite sides of the No. 6 position, the axis of symmetry, we are clearly dealing with a reversal of asymmetry which most probably occurs in the neighborhood of the No. 6 feather of the transverse sequence.

TABLE III

SHAFT BARB NUMBERS, FEATHERS 3, 6, AND 9 OF TRANSVERSE BREAST SEQUENCE

| *c* | Feather 3 | | | Feather 6 | | | Feather 9 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *d* | *l* | *r* | *d* | *l* | *r* | *d* | *l* | *r* |
| 1 | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 2 | 0.64 | ...... | ...... | 0.45 | ...... | ...... | 0.64 | ...... | ...... |
| 3 | 1.28 | 2.0 | 1.5 | 1.12 | ...... | ...... | 1.57 | 1.0 | 1.0 |
| 4 | 1.92 | 2.7 | 2.4 | 1.95 | 0.7 | 0.7 | 2.69 | 2.3 | 3.0 |
| 5 | 2.88 | 4.3 | 3.8 | 3.36 | 3.0 | 3.4 | 4.32 | 4.7 | 7.0 |
| 6 | 4.16 | 6.6 | 5.7 | 4.70 | 5.2 | 6.0 | 7.08 | 9.0 | 13.0 |
| 7 | 5.77 | 10.0 | 8.3 | 5.82 | 7.0 | 8.2 | 9.79 | 15.1 | 19.8 |
| 8 | 7.69 | 14.7 | 12.2 | 7.97 | 11.4 | 12.5 | 14.34 | 24.5 | 30.7 |
| 9 | 10.24 | 21.0 | 17.6 | 10.88 | 18.3 | 19.0 | 18.01 | 34.4 | 40.0 |
| 10 | 13.77 | 30.5 | 25.7 | 13.50 | 25.0 | 25.3 | 22.34 | 46.0 | 51.3 |
| 11 | 17.92 | 42.3 | 37.0 | 16.38 | 32.8 | 32.3 | 27.42 | 59.3 | 64.7 |
| 12 | 22.72 | 56.0 | 51.0 | 19.52 | 41.2 | 41.0 | 32.70 | 73.0 | 77.5 |
| 13 | 27.74 | 68.5 | 65.0 | 23.42 | 52.7 | 52.0 | 39.68 | 91.0 | 94.0 |
| 14 | 31.55 | 78.0 | 75.5 | 27.04 | 62.7 | 62.0 | ...... | ...... | ...... |
| 15 | ...... | ...... | ...... | 30.82 | 73.0 | 73.0 | ...... | ...... | ...... |
| 16 | ...... | ...... | ...... | 34.40 | 84.0 | 83.5 | ...... | ...... | ...... |
| 17 | ...... | ...... | ...... | 37.44 | 93.0 | 92.0 | ...... | ...... | ...... |

Curves representing the marginal barb numbers are possibly a more direct measure of reversal of asymmetry than are curves for shaft barb numbers. It is to be noted that the differences exhibited by the two sets of curves are in every case more marked for marginal barb numbers than for barb numbers at the shaft intercepts.

*b*) *Barbs on c-isochrones.*—Table IV gives the number of barbs on successive c-isochrones applied to feathers 3, 6, and 9 at the distances indicated from the apex of each feather. The number of barbs thus defined by c-isochrones for the feather from the third row are shown in text Figure 4, curves $N_c$, $r$ and $l$, representing right and left vane-halves. The corresponding curves for feather 9 are shown in text Figure 5. The number of barbs on successive c-isochrones in the right vane-half increases very rapidly during the initial stages of barb formation; the curves then fall for both vane-halves, and the number of barbs in the right collar limb becomes in the instance of feather 9, text Figure 5, less than the number in the left collar limb at about 7 mm. axial growth of the germ. The number of barbs in the left collar complement remains definitely above the number in the right collar limb after the curves have crossed.

## TABLE IV

BARBS ON C-ISOCHRONES, FEATHERS 3, 6, AND 9 OF TRANSVERSE BREAST SEQUENCE

| *c* | FEATHER 3 | | | FEATHER 6 | | | FEATHER 9 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *d* | *l* | *r* | *d* | *l* | *r* | *d* | *l* | *r* |
| 1 | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... | ...... |
| 2 | 0.64 | ...... | ...... | 0.45 | ...... | ...... | 0.64 | ...... | ...... |
| 3 | 1.28 | 30.7 | 27.7 | 1.12 | ...... | ...... | 1.57 | 18.4 | 32.5 |
| 4 | 1.92 | 33.7 | 31.8 | 1.95 | 36.5 | 33.9 | 2.69 | 28.7 | 40.0 |
| 5 | 2.88 | 37.5 | 38.9 | 3.36 | 44.6 | 41.8 | 4.32 | 39.3 | 44.0 |
| 6 | 4.16 | 42.4 | 46.3 | 4.70 | 49.8 | 46.0 | 7.08 | 48.0 | 47.8 |
| 7 | 5.77 | 46.6 | 50.7 | 5.82 | 53.3 | 48.8 | 9.79 | 54.6 | 52.0 |
| 8 | 7.69 | 50.1 | 55.8 | 7.97 | 59.3 | 53.7 | 14.34 | 62.5 | 55.3 |
| 9 | 10.24 | 54.0 | 59.6 | 10.88 | 63.5 | 58.0 | 18.01 | 65.8 | 59.0 |
| 10 | 13.77 | 57.5 | 66.3 | 13.50 | 65.7 | 61.4 | 22.34 | 70.0 | 60.7 |
| 11 | 17.92 | 58.9 | 70.0 | 16.38 | 67.2 | 63.3 | 27.42 | 71.7 | 61.3 |
| 12 | 22.72 | 60.0 | 71.0 | 19.52 | 68.8 | 64.8 | 32.70 | 74.0 | 61.0 |
| 13 | 27.74 | 59.5 | 70.0 | 23.42 | 68.8 | 66.0 | 39.68 | 75.0 | 62.0 |
| 14 | 31.55 | ...... | ...... | 27.04 | 69.3 | 65.2 | ...... | ...... | ...... |
| 15 | ...... | ...... | ...... | 30.82 | 70.0 | 65.0 | ...... | ...... | ...... |
| 16 | ...... | ...... | ...... | 34.40 | 68.0 | 64.0 | ...... | ...... | ...... |
| 17 | ...... | ...... | ...... | 37.44 | 67.0 | 64.0 | ...... | ...... | ...... |

The rate at which the right collar complement, feather 9, text Figure 5, is established is much greater than the rate at which the barb complement of the left collar limb is completed. Nevertheless, during the formation of the main part of the vane of feather 9, the number of barbs in the left collar is considerably greater than in the right collar limb.

Curves for the number of barbs in the collar limbs of the third feather in the transverse row (text Fig. 4) are the reverse of those shown by feather 9, i.e., the number of barbs in the left collar limb increases more rapidly during the initial stages of axial growth, and at later stages the right collar limb comes to have the greater number of barbs.

Table IV shows that the number of barbs in the collar of the feather from the sixth position is almost perfectly symmetrical on corresponding c-isochrones. This is, in fact, evident from the data shown in Tables II and III, since marginal and shaft barb numbers on successive isochrones are almost uniform in the two vane-halves.

It is not certain just what significance should be attached to the number of barbs on opposed c-isochrones, since we do not know the exact relations which obtain between number of barbs, size of barbs, and possible changes in the absolute length of collar between the ventral triangle and the shaft primordium. But it is certain, nevertheless, that we are dealing here also with well-defined phases of asymmetry. This is further borne out by the remarkable manner in which reversal of the relations of asymmetry in feathers 3 and 9 is shown in the initial stages of growth (i.e., the first 3 or 4 mm. of axial growth) as well as in the later stages of more or less complete barb complements. The reversal of symmetry relations is definitely shown at all comparable levels of feathers 3 and 9.

*c) Relative ratios in marginal barb numbers.*—We have shown elsewhere (Juhn and Fraps, 1936) that the barb numbers in opposite vane-halves may be compared through the main vane structure according to the formula $y=bx+a$. If we plot the number of barbs formed at successive isochrones in the opposite vane-halves as abscissas and ordinates, the data describe a straight line for opposite vane-halves through at least the main portion of the vane.[2] In text Figure 6 we give the data for feathers 3 and 9, marginal intercepts only, thus plotted (data from Table II). Abscissas represent the marginal barb numbers in left vane-halves; ordinates represent marginal barb numbers at corresponding levels in the right vane-half.

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[2] We use this formula in the present connection purely as a mode of description. See Juhn and Fraps, 1936.

It is evident from Table II that the relative ratio of marginal barb numbers is practically unity in the vane-halves of feather 6; from the same table it is evident that marginal numbers increase in feather 9 more rapidly on the left side, while in feather 3 the more rapid increments are on the right side. We have determined by graphic methods the slope of the line through the region of constant relative marginal ratios for each of the three feathers. The equations are as follows:

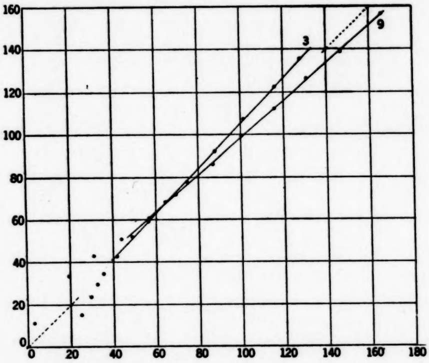

FIG. 6.—Relative ratios in marginal barb numbers, feathers 3 and 9 of transverse breast sequence. Abscissas represent (for each feather) barb numbers in left vane-half; ordinates, barb numbers in right vane-half at corresponding c-isochrone intercepts. In a perfectly symmetrical feather, barb numbers would be equal in left and right vane-halves at all points; the slope of the line through these points would be unity.

Feather 3: $y = 1.07x - 2.$

Feather 6: $y = 0.98x - 2.$

Feather 9: $y = 0.87x + 11.$

A value of $b = 1$ in these results would mean, of course, that no differentials are present. In feather 3 the relative marginal ratio is practically constant between barbs 50 and 130. In the longer feather (No. 9) the ratio is approximately constant from the fiftieth barb through formation of the $165^{th}$ barb. We have not given the curve for feather 6; it is evident from the formula that the differences are very small.

It should be emphasized that the data plotted in text Figure 6 represent relative ratios in a definite "serial" process, i.e., the rates at which apical barb loci are "defined" as determined by application of the c-isochrone. The straight lines drawn through the determined points show beyond doubt that the given formula adequately describes the relation between these magnitudes in the opposite vane-halves of the feather. It might be objected that the differences actually shown by these feathers are small; but it should be realized that all asymmetries in the breast tracts are of relatively small degree, as compared, for example, with feathers of the wing tracts.

We cannot at present definitely evaluate the curves shown in text Figure 6 either in terms of growth or differentiation. But as we have attempted to emphasize in connection with all of the data presented here, the important relation is that a definite reversal of sign or magnitude characterizes feathers 3 and 9 in all c-isochrone measures. Relative ratios in marginal barb numbers are probably the most direct measure of this reversal that we have been able to obtain.

Application of the c-isochrone to the feathers of the transverse sequence dealt with here shows by all measures or "indexes" that feather 6 is fairly symmetrical, and that reversal of asymmetry in feathers 3 and 9 occurs in the order expected on the basis that the No. 6 position represents in fact the axis of symmetry in the tracts.

### 3. GROWTH-RATES OF REGENERATING FEATHERS ON TRANSVERSE CO-ORDINATES

We include these data here although as noted earlier the rate of growth of regenerating feathers on a transverse sequence must be compared for definite periods in the regenerative cycle.

Curves representing the growth increments of two transverse sequences (on opposite sides of the bird's body) are given in text Figure 7. The positions of feathers in mediolateral order are given at the base of the figure; ordinates represent simultaneous lengths of regenerating feathers, adjusted to a 5-mm. initial length in order to eliminate differences in times of emergence.

The topmost curves show, in both sequences, maximum lengths in the region of the axis of symmetry; we cannot say, however, that there is a well-defined "peak" at the No. 6 position. Nor are growth increments uniformly greater in the region of the axis of symmetry: at approximately 20 mm. in length, maximum rate of growth appears to characterize positions medial to the No. 6 position.

Correction of these data by reference to the "true" transverse co-ordinates of the breast tracts would accentuate growth increments in the region of the axis of symmetry. The differentials represented are therefore to be taken as minimal for these particular sequences.

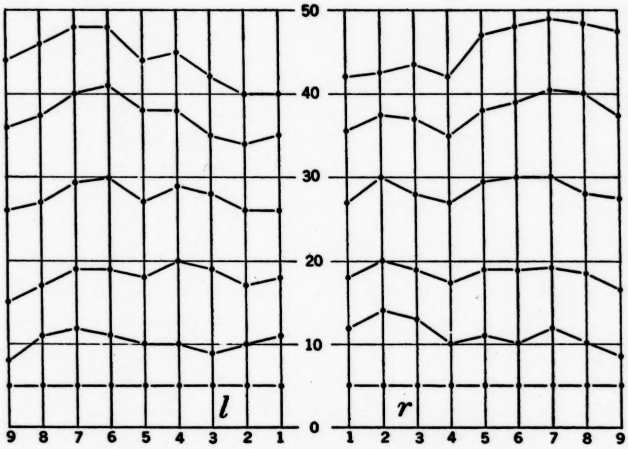

FIG. 7.—Growth increments in regenerating feathers of transverse sequences, breast tracts. Abscissas, mediolateral order of left and right sequences; ordinates, lengths of regenerating feathers. Each pair of curves (right and left tracts) represents the lengths of feathers at the same time, adjusted on basis that all feathers are 5 mm. in length at time of first determinations.

We are clearly dealing here with a complex situation—certainly one that cannot be reduced to the orderly relations describing total lengths of feathers on the transverse sequences or the relations of reversed asymmetry as measured by c-isochrone magnitudes. We shall accordingly treat these results with some reservations at this time.

### C. POLYPHASIC FUNCTIONS OF THE TRANSVERSE CO-ORDINATES

The weights of regenerated feathers lying along transverse sequences show a striking discontinuity when compared with the data presented under the foregoing orders. Text Figure 8 represents average weights of feathers for six rows. The unbroken curve is the average length of regenerated feathers 1–9 for the same transverse sequences. As we have shown earlier, the length of feathers increases continuously across the tract. In feathers 1–6 of the transverse sequence, the total weight of the completely regenerated feather is almost directly proportional to the length of the feather. Weights from the sixth to the ninth feather, however, show the curve to break sharply; and feather 9 shows a definite decrease in total weight, although length of the completely regenerated feather is greatest for the sequence.

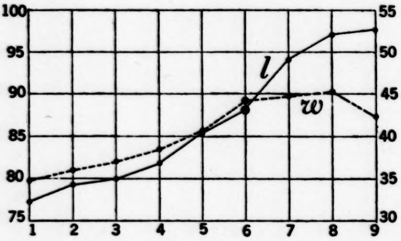

FIG. 8.—The relation between length and weight of regenerated feathers from six transverse sequences of the breast tracts. Abscissas, positions of follicles in mediolateral order; left ordinates, length of the regenerated feathers in millimeters; right ordinates, weight of the same feathers in milligrams. Curve *l*, length of feathers; curve *w*, weight of feathers.

If, as we have suggested earlier, the length of completely regenerated feathers may be treated as a continuous function, while those properties of feathers which show a true reversal of asymmetry are treated as discontinuous functions, we might interpret the data on weight of regenerated feathers to represent a partial relation between these two determinative functions. The exact character of this possible relation need not be discussed here. It should be emphasized that we use the term "polyphasic" to describe an actual incongruity of relation, and for the moment we need not attach too much significance to the term.

We summarize the experimental results under the following three headings, which are referable to the observations presented under the notation of continuous, discontinuous, and polyphasic functions:[3]

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[3] According to Greenwood and Blyth (1935*b*), asymmetrical pigmentation reactions

1. Properties of the transverse sequences which change continuously through the tract in mediolateral order.
2. Properties of the transverse sequences which change continuously from feather 1 to feather 6, and show definite reversal from feather 6 to feather 9.
3. Properties of the transverse sequences which show a definite relation, direct or inverse, through one limb of the symmetry orders (feathers 1–6) but are not so related in the other limb of the order (feathers 6–9).

## III. THE DISTRIBUTION OF PLUMAGE PROPERTIES AS FIELD FUNCTIONS

The relations established in the first part of this paper involve, as a matter of course, the concept of gradients or fields. We are concerned here primarily with transverse co-ordinates, and particularly with the fact that the properties of individual follicles on these co-ordinates are referable to two distinct anteroposterior axes: we recognize therefore two "incongruous" transverse gradients.

Since we interpret the relations recorded in this paper as "gradient" or "field" relations, we shall begin with a statement of what we understand by these terms. We shall not attempt a critical examination of the many concepts of fields and gradients which have appeared in the literature. Discussions on these lines have come recently from Huxley (1935) and Weiss (1935).

The most comprehensive experimental analysis and theoretical development of relations implicit in either the gradient or the field concept is that of Child, and we wish to emphasize that we have used the term "field," rather than "gradient," in view of the two-dimensional gradient vectors of the plumage tracts. The analytic datum remains the gradient or physiological axis within the space of the

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may be induced in the breast tracts independently of either of the transverse orders which we describe here. The distribution of asymmetries effected by microinjection of female hormone is referable to the site of injection as a center of diffusion, and Greenwood and Blyth conclude that the action of the hormone is "a direct one." If this is true, it is clear that reactions of such an order cannot involve directly or entirely the primary asymmetry differentials of the germ. The results described by Greenwood and Blyth bring up interesting questions concerning the mechanism of transport and distribution of the hormones, but these need not concern us further here.

field; and the gradient concept as this has been developed by Child is from this point of view the most representative characterization of the primary differentials composing the field that has yet appeared.

In the present discussion of fields we shall attempt to avoid two common implications. The first is that the *field* represents a "general" and more or less indefinite set of relations rather than exact and limited relations. If functions referable to a gradient or a field are exact, e.g., the properties of individual feather follicles composing a transverse sequence, it is logically necessary to assume that the field energy which determines (or has determined) those properties and their distribution must also be (or have been) equally precise in distribution and limited in specific action. This does not mean that the distribution of field energies or gradient differentials is an invariable relation, a point sufficiently evident in view of the ease with which gradient differentials are subject to modification.

The second implication in the concept of fields is that of "ultimate" explanatory values without due reference to the fundamental properties and characterization of the dynamic field particularly and in its simplest physical sense. That some primary distinction must be made between fields is sufficiently evident in the recognition of direct chemical action as a definite phase of "organizer" action, distinct from the dynamic rôle of the "individuation field" (Waddington and Schmidt, 1933). The transmissive (energy) nature of gradient (and *ipso facto* field) differentials of a *primary order* recognized earlier by Child (1928 *et ante*) is implicit in Waddington and Schmidt's distinction, and likewise in Huxley's (1935) "distance" and "contact" organizers.

### A. ENERGY AND RESIDUAL FIELDS

In a strictly dynamic sense, a field is an orderly distribution of energy in space. The distribution of the field energy is not homogeneous, i.e., it is not uniform at every point within the space of the field but is subject to differentials in *intensity* and in *direction;* it is these differentials which we evaluate, directly or indirectly, in defining a field.

A *point* in a purely dynamic field is completely defined by the intensity and direction of the field energy at that point, and the *field* is completely defined in terms of the intensity and direction of the field energy at all points within the space of the field. In the classical representation of Faraday the (electromagnetic) field is conceived in terms of lines of force: the direction of the lines at a point is the direction of the field energy at that point, and the density of the lines represents the intensity of the field at that point. Biologically, of course, the problems presented by field energies are "unique" in two respects: specificity of energy and specificity in effect.

The distribution of forces within the space of a strictly physical field will be symmetrical with respect to defined loci ("centers," axes) only if the space of the field (or the distribution of bodies within the space of the field) is uniformly or symmetrically "permeable" to the field energy. "Distorted" or "asymmetrical" fields[4] are not, therefore, *a priori* evidence for a corresponding distortion or asymmetry in distribution of the same field energy as an inherent property of the energy itself. It is therefore necessary to distinguish in the morphogenetic process "apparent" and "real" energy fields.

1. A (persistent) dynamic field involves the presupposition of energy relations which are maintained with reference to centers or axes from which there are definite and exact decrements in the field energy. The experimentally imposed physiological gradients of Child (1924, 1928) are, in their simplest form, examples of dynamic field energies from this point of view.

2. A temporary dynamic field may determine once and for all a set of relations which remain constant subsequent to the *determinative* action of the field. The determined properties, or relations, will thenceforth not require the continued action of the primary energy field for the expression of differentials. We shall refer to the composite differential properties of any such "apparent" field as a *residual* field. Putting the relation a little differently, and with the properties of plumage tracts as example, we define a residual field as the order of differentials imposed by a dynamic field upon a series or

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[4] The peculiar weight curves for feathers composing a transverse sequence may be significant in this connection, on the assumption that the "forces" referable to the No. 6 position must operate in a substrate with previously established heterogeneous properties, i.e., "lower" toward the No. 1 position, "higher" toward the No. 9 position.

aggregate of individual "points" or mechanisms, and thenceforth maintained independent of the morphogenetically effective energy.

Dynamic properties (e.g., growth-rates), may characterize residual fields. The existence of such properties is not, in itself, evidence that a dynamically effective causal energy is the "source" of differentials exhibited by the "points" or mechanisms composing the manifold or aggregate, e.g., the plumage tract. The dynamic phase in the morphogenetic process may, at least theoretically, be strictly limited to the *determination* of the properties of differing points or mechanisms within the original space of the field. Once determined, the several points or mechanisms must show differential potentialities or properties—"dynamic" or otherwise—which are field functions in a strictly residual sense.

If these propositions have been made clear, it follows that we have at least two distinct possibilities in the definition of all apparently dynamic fields in the organism. The first is that the field may represent a true energy field in the strict sense that differentials between points within the space of the field are dependent upon a continued or "persistent" distribution of a field energy. The second is that a temporary energy field may bring about definite determinations upon, or configurations within, masses (bodies, e.g., genes) within the space of the field. The initial transforming field energy need not, in this instance, be maintained; its dynamic function is in this sense strictly that of determination.[5] Differential properties, potentialities, or intensities exhibited by individual aggregates thus determined, compose residual fields in the limited sense that we apply that term to the plumage tracts.

### B. DISCRETE TERMS IN THE MORPHOGENETIC PROCESS

The ideal measure of a dynamic morphogenetic field, as of any "true" field, would be the absolute intensity of the field energy at a sufficient number of points within the space of the field to permit of its complete mathematical formulation. In the absence of so elegant a measure of even the simplest type of field or gradient differential in the organism, we can only determine the distribution of *properties* which represent either direct field variables *or residuals* of an assumed primary field energy.

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[5] We use the term "determination" here strictly with reference to the properties of individual follicles described earlier; if these properties are, in fact, residuals referable to previously established (however temporary) dynamic field differentials, determination in the limited sense that we employ the term here refers to the specific *transformation* in the reactive substrate which shall thenceforth define or characterize the substrate (and its derivatives) independently of antecedent dynamic field energies. We might say, somewhat schematically, that residuals represent *determinations* and that differentials in the initial field energy represent *potentials*.

Theoretically at least, the properties of the plumage tracts which we consider here may reside in genes or in specific collocations of cells composing the mechanism of the germ. It seems probable that we are dealing with what may be called "mechanisms" rather than specific quantitative variables in individual genes. These individualized entities or mechanisms comprise the feather germ and all of its potentialities.

In attempting to apply the conception of residual fields to the breast tracts, we must remember that we are dealing with two apparently independent sets of gradient or differential properties at right angles to the anteroposterior axis of the tract. The data show clearly that two variables across the transverse sequence of the breast tracts are not commensurable, i.e., we cannot reduce the data to functions of a single variable expressing changes in the entire transverse sequence.

Our interpretation of the results presented in this paper is then to consider the "incongruous" properties of the transverse sequences to be residual functions of at least two discrete field energies. We may denote each of the two transverse gradient orders (describing continuous and discontinuous properties) as discrete gradient or field orders, and the determination of the properties characterizing each of these gradient orders may accordingly be described as a discrete term in the morphogenetic process.

It follows from this interpretation of the data presented in this paper that at least one of the transverse field differentials need not be effective within the tract as a dynamically determinative and persistent gradient (cf. Huxley and De Beer, 1934). An alternative to this view is that the two differential field functions of the breast tracts represent qualitatively *specific energies* which are continuously (or persistently) effective in the determination of the properties exhibited by the individual regenerating follicle. The possibility that dynamic differentials of this character actually determine the properties of the individual follicle is at least made highly improbable by the transplantation experiments of Danforth and Foster (1929), although Danforth's (1935) mosaic patterns are possibly due to "persistent" field energies. If, however, one of the determinative differentials of the breast tracts is supposed to be the expression of a fixed, previously determined property of the individual mechanism, there is no good reason why we may not apply similar considerations to all properties exhibited by the follicles of the tract unless we have direct evidence to the contrary. This is only to say that we suppose all properties of the follicles of the breast tract to be embryologically determined and that the residual gradient functions represented in the regenerative properties of individual follicles are evidence for dynamic field orders in the morphogenetic process.

The theoretical significance of the interpretation of fields which we have applied to the plumage tracts can be summarized briefly. We suppose, in the first place, that one definite function of the morphogenetic field is to define discrete properties of individual cells, or of individual mechanisms, e.g., feather follicles. We assume, in the second place, that more than one set of field differentials (energy fields) may operate upon the same individual cell, or collocation of cells, and determine subsequent orders of field residuals which may or may not coincide with spatial co-ordinates of previous residuals.

In the instance of feather regeneration in the breast tracts, the residual properties are evident as quantitative differentials in regenerative capacities or intensities with respect to the mid-ventral axis of the bird's body or the axis of symmetry within the tract.

This concept of the relation between the dynamic field and its residuals[6] simplifies some, at least, of the difficult problems posed by the field concept when the field energy is considered to be a constantly effective (persistent) energy. On theoretical grounds it does not seem necessary to postulate so complex a relation of differentially effective energies as is seemingly required by this view. The concept of discrete terms in field transformations, thenceforth evident as residuals, is a logical alternative in any event.

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[6] It is at least theoretically possible that the "individuation" field of Waddington and Schmidt (1933) represents a purely dynamic field, dependent upon and evident as energy relations. The chemical "organizer," on the contrary, is clearly a residual in the sense that we have defined that term. But there is a definite distribution of the organizer (speaking now of the "chemical") within the tissues of the embryo, and in our opinion the order of distribution of the organizer can only be accounted for in the end by reference to rigorously dynamic (or energy) fields. This view has been repeatedly emphasized by Child (see particularly 1924 and 1929) in the same and other connections, but its prime significance has been all too frequently obscured by loosely defined interpretation of gradient and field concepts.

In view of the theoretically limited time interval involved in the postulated effective or determinative action of dynamic fields from this point of view, we may refer to such fields as *excitation fields*. An excitation field energy, effective for a short time, but attaining a definite and exact end during that time, is at least a theoretically possible statement of the manner in which genetic differentials may be established. The results given in the first part of this paper suggest that specific genetic compositions may be directly referred to field functions from this point of view.

## IV. Summary

1. Analysis of the distribution of properties of individual follicles composing transverse series of the breast tracts (i.e., mediolateral series) gives evidence for the existence of two (independent) gradient orders.

2. These results are discussed as evidences for residual field or gradient functions, and the theoretical relation of excitation and residual fields (or gradients) in the morphogenetic process is briefly touched on.

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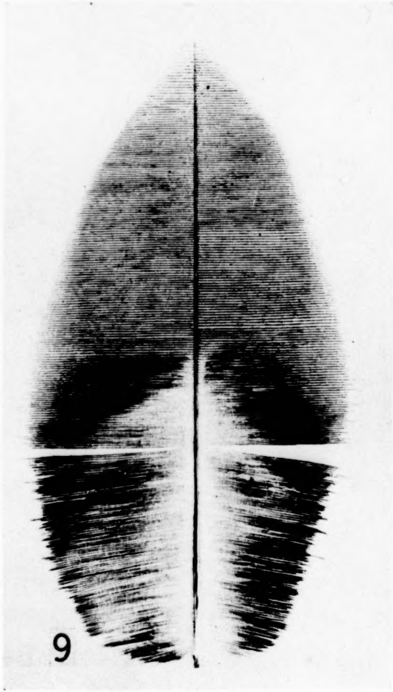
# PLATE I



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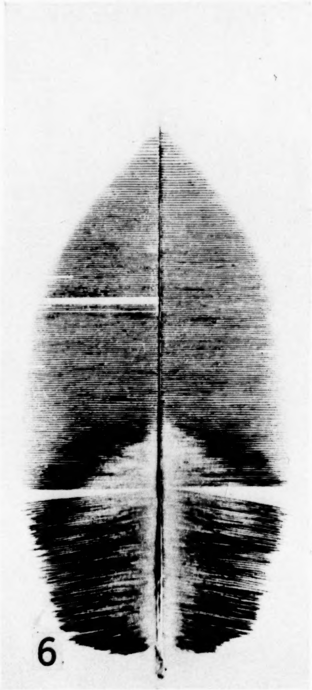

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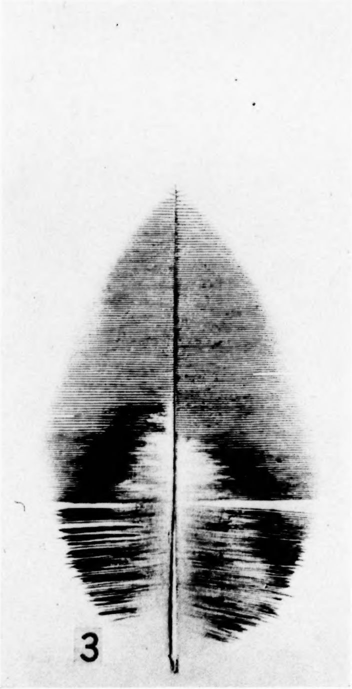

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## PLATE I

Photographs of feathers from the third, sixth, and ninth positions of a transverse sequence, left breast tract, Brown Leghorn capon. Feather 6 comes from the approximate axis of symmetry of the tract. Feathers 3 and 9 are each three positions removed from the axis of symmetry. The increasing length of the series (1–9 in mediolateral order) is evident in the lengths of the three feathers reproduced here. Notice also the reversal of symmetry relations in apical contours, in lengths of opposed barbs, and in the asymmetrical distribution of fluff in feathers 3 and 9. In all these characteristics, feather 6 is practically symmetrical.